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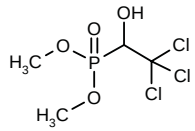
Metrifonate

Rec INN

Treatment for Alzheimer's Disease
Acetylcholinesterase Inhibitor

Trichlorfon (USAN)
Metriphonate (BAN)
Bay-a-9826
Bayer-L-1359
Bilarcil®
Dipterex®
Dylox®
Neguvon®

(2,2,2-Trichloro-1-hydroxyethyl)phosphonic acid dimethyl ester



$C_4H_8Cl_3O_4P$ Mol wt: 257.44

CAS: 000052-68-6

EN: 163528

Synthesis

Metrifonate is easily synthesized (1) in a one-step spontaneous exothermic condensation of equimolar amounts of chloral hydrate with dimethylhydrogenphosphite (Scheme 1), which in turn is accessible by reacting PCl_3 with methanol. Contrary to similar reactions described earlier (1950-1952) in two Soviet papers and a U.S. process patent, no catalyst is needed if the aldehyde compound is chloral. Therefore, metrifonate can be readily crystallized directly from the reaction mixture.

Description

White crystals, m.p. 83-4 °C, d420 = 1.73, nD20 = 1.3439. Solubility at 25 °C: water 154 g/l, $CHCl_3$ 750 g/l, ether 170 g/l, benzene 152 g/l, very slightly soluble in pentane and hexane. At 37 °C, metrifonate is about equally stable in bicarbonate buffer (pH 7.6) and phosphate buffer (pH 7.4) ($t_{1/2}$ = 1.5 and 2.6 h, respectively).

Introduction

Even in today's era of rational drug design dominating psychopharmacology, there are high-potential compounds that have what one might call a long and interesting history. Prominent examples can be found in the class of acetylcholinesterase inhibitors for the palliative treatment of Alzheimer's disease: physostigmine (Synapton®), tacrine (Cognex®) and galanthamine (Nivalin®/Reminyl®) all had long clinical records before anybody considered them candidates for drug development to treat the symptoms of Alzheimer's disease.

In terms of historical convolutions, however, none of these molecules is a match for metrifonate, an organophosphorus compound that was introduced by Bayer as an insecticide in 1952. Based on its reaction against some helminths in cattle which had been noted from 1955 onward, metrifonate was proposed as an antiparasitic drug (Bilarcil®) for the treatment of schistosomiasis and hookworm infection in 1960 (2), although it was not before 1965 that Bayer became actively involved in the trials. The chronology of these early developments, and the strong involvement of the WHO, has been reviewed (3). Although newer drugs such as praziquantel have broader activity in all forms of schistosomiasis, a course of three doses of metrifonate (7.5-10 mg/kg) in fortnight intervals still is considered standard therapy for the urinary variant caused by *S. haematobium* bladder vessel infestation. The WHO has therefore retained metrifonate on its list of essential drugs.

It appears that a "second use" indication for metrifonate concerning the symptomatic treatment for Alzheimer's disease was first considered in the late

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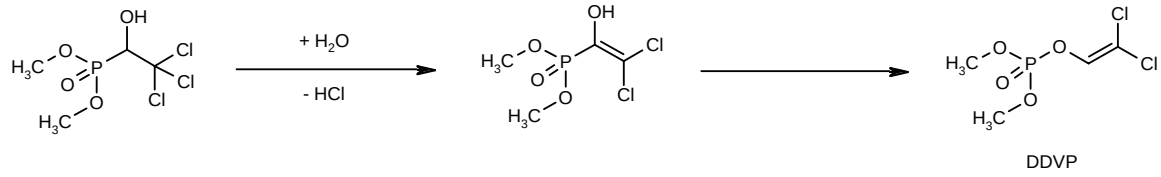
Scheme 1: Synthesis of Metrifonate

Scheme 2: Decomposition of Metrifonate



1980s. Amazingly, this concept - which should have been quite obvious since at this time similar patents had been granted for two other well-known acetylcholinesterase inhibitors, tacrine and galanthamine - was put forward not by Bayer but rather by two reputed dementia researchers affiliated with the University of Southern Illinois, Robert E. Becker and Ezio Giacobini. These scientists had suggested earlier that "a 'slow-release formulation' may be necessary to achieve increases in acetylcholine concentrations that will have therapeutic effects in senile dementia of the Alzheimer's type" (4). The patent (5), issued in 1990, names the University Board of Trustees as an assignee.

Pharmacological Actions

Metrifonate is totally unique among the acetylcholinesterase inhibitors currently used or proposed for the treatment of Alzheimer's disease because it is an inactive prodrug. The active compound dichlorvos, (2,2-dichlorovinyl dimethylphosphate; DDVP), is formed in a nonenzymatic reaction by hydrolysis and elimination of HCl followed by rearrangement of an intermediate (6) according to Scheme 2.

Dichlorvos had long been used as an insecticide in products such as Vapona®, Fly-Die®, Didivan® and others. It is a potent (up to 96% inhibition) inhibitor of vertebrate brain cholinesterases that rapidly leads to accumulation of acetylcholine in most brain sections containing cholinergic nerve terminals (7, 8). Evidence for its *in vivo* generation from metrifonate had mostly been indirect until 1978, when it was demonstrated that intraperitoneal administration of 125 mg/kg metrifonate to mice resulted in DDVP formation in the brain, where it achieved a 70% inhibition of acetylcholinesterase, and a corresponding increase in acetylcholine concentrations, within 15 min (9). In rat brain, the aqueous-soluble and detergent-soluble forms of acetylcholinesterase were almost equally inhibited (10).

Metrifonate (and hence, DDVP) was originally thought to exert almost exclusively cholinergic effects (11), which might have explained why a higher degree of cholinesterase inhibition was tolerated with metrifonate than with physostigmine or tacrine (12). In contrast, later studies in rats have shown that it significantly elevated dopamine and norepinephrine (but not serotonin) levels in rat brain (13). However, these effects were obviously indirect ones as both metrifonate and DDVP did not significantly bind to neurotransmitter receptors (14). ED_{50} values for cholinesterase inhibition in rat blood and brain were 80 and 90 mg/kg, respectively; if animals were deprived of food for 18 h before drug administration, these values dropped to 45 and 60 mg/kg. Also, drug sensitivity was higher in young (3 months age) than in older (19 months age) rats. Regardless of age and satiety status, cholinesterase inhibition in brain was strongly correlated with plasma inhibition during the maximum effect phase of the drug (15).

In human cortex preparations, metrifonate inhibited butyrylcholinesterase (which has no known physiological role in the CNS compartment) more than acetylcholinesterase (16). Based on the experience with other such inhibitors, this might be of little clinical relevance.

Similar to other cholinesterase inhibitors, metrifonate and DDVP also had indirect central effects by their action

on the cholinergic system, such as increase in local cerebral glucose utilization (17) and neocortical EEG arousal (18) in rats as long as the nucleus basalis region (a center of cholinergic innervation) was intact.

As a consequence of the "grandfather drug" status of metrifonate obtained in an application totally different from Alzheimer's disease, preclinical work on its cognitive effects was carried out more or less in parallel with phase II and III studies, and has been reported only very recently.

In aging rabbits, metrifonate improved acquisition of eyeblink conditioning (a model for associative learning) at erythrocyte acetylcholinesterase inhibition rates of 30-80% below baseline, with maximal behavioral efficacy at 40% inhibition (19). In rats that were compromised in their central cholinergic functioning by old age, lesioning of the medial septum or pretreatment with the muscarinic acetylcholine receptor antagonist scopolamine, spatial navigation in the water maze and passive avoidance behavior were improved by oral doses of 10-100 mg/kg (20, 21). These effects of acute metrifonate administration were sustained during subchronic treatment with 30 mg/kg for 3 weeks (22). In basal forebrain-lesioned rats, acetylcholine discharge was increased under these conditions (23, 24) but the effects of hippocampal damage by corticosterone could not be reversed (25). Interestingly, this enhancing effect on cognition was also seen in young and adult intact rats, with a bell-shaped dose-response curve and optimal doses of 10-30 mg/kg (26), causing the authors to speculate whether additional unknown mechanisms of drug action might be present.

Indications for a limited synergy between metrifonate and the calcium channel blocker, nimodipine, have been obtained (27-29).

Pharmacokinetics and Metabolism

Because of its prodrug character, metrifonate acts as a *de facto* slow-release formulation for its cholinesterase inhibitor rearrangement product, dichlorvos. It inactivates cholinesterases noncompetitively by irreversible phosphorylation at critical positions (30); the observed recovery rate of enzymatic activity therefore essentially reflects de novo synthesis of these enzymes.

After intraperitoneal administration of 125 mg/kg metrifonate to mice, DDVP was detectable in the brain within less than 15 min (9). Intramuscular administration of 80 mg/kg to rats reduced the activity of brain cholinesterase to 26% at 30 min, followed by a recovery to 50% at 180 min and a return to 74% at 360 min. Levels of acetylcholine increased by 45% at 45 min, then returned to normal by 120 min. When metrifonate (2.5 mg) was given intracerebroventricularly the activity of cholinesterase decreased at 30 min to 20% in hippocampus, 22% in the medulla, 50% in the cerebellum, 58% in the striatum and 72% in cortex.

Correspondingly, levels of acetylcholine increased maximally at 45 min in hippocampus and cortex and peaked in the striatum at 60 min. The greatest increases were seen in the hippocampus and cortex with 60 and 55%, respectively. This *in vivo* response profile differed significantly from that obtained with physostigmine under similar conditions (31, 32).

In man, metrifonate was readily absorbed and reached peak levels in blood within 2 h after single oral dosing with 2.5, 5.0, 7.5 or 15.0 mg/kg. At any given time, DDVP concentration was about 1% of the amount of metrifonate in plasma and 0.5% or less in erythrocytes. Butyrylcholinesterase activity in plasma reached very low levels within 15 min and remained inhibited for more than 8 h. Inhibition of red blood cell cholinesterase, which is often used as a surrogate measure for brain acetylcholinesterase, was inhibited by only 60-80% under these conditions, in a dose-dependent fashion (33, 34). Specifically, after a single oral dose of 7.5 mg/kg, metrifonate was absorbed with a C_{max} of 50.5 ± 18.9 μmol/l, obtained between 0.17 to 1 h after drug ingestion. Whole blood half-life, oral clearance and AUC were 2.07 ± 0.24 h, 0.34 ± 0.06 l/h/kg and 89.2 ± 16 μmol.h/l, respectively (means from 6 healthy individuals) (35). Replacing the original GC/MS method (36) for determination of metrifonate and dichlorvos in plasma with modified GC-based methods capable of detecting the compounds in the micromolar to high-nanomolar range, respectively (37), metrifonate was detectable for up to 8 h but dichlorvos concentrations had fallen below the levels of determination by this time.

Using an HPLC method with UV detection that pushed the detection limit for metrifonate and DDVP to 1 μg/ml and 40 ng/ml, respectively (38), Unni *et al.* conducted two additional studies on pharmacokinetics and pharmacodynamics in the target population, Alzheimer's patients (39). In the first study, 3 patients with prior exposure to the drug received oral metrifonate (7.5 mg/kg). Plasma cholinesterase inhibition peaked to 78.5 ± 12.3% at 15 min, while maximum erythrocyte cholinesterase inhibition seen at 1 h was 61.0 ± 11.0%. Plasma inhibition was unchanged at 6 h, whereas the RBC acetylcholinesterase enzyme recovered with a half-life of 7.0 ± 3.5 h. In the second study, 6 metrifonate-naive patients and 6 controls exhibited a mean half-life of 2.3 ± 0.3 h in plasma, with cholinesterase recovery $t_{1/2}$ of 9.0 ± 3.3 (plasma) and 26.6 ± 15.2 days (RBC) after 7.5 mg/kg oral metrifonate. The latter finding confirmed earlier data indicating that the effective half-life of overall cholinesterase inhibition is 5-6 days (40), giving the clinically unique option of weekly administration. In addition, DDVP (but not metrifonate) is suitable for transdermal delivery (41), although development of this route of administration might not be urgently required.

Clearance of metrifonate in man occurs primarily via dichlorvos excreted in urine; however, a small fraction is oxidatively metabolized by pH-dependent reactions yielding products with inhibitor activity (9). Such compounds include semidemethylated metrifonate or DDVP, monomethylphosphate and dimethylphosphate. It has been claimed (42) that the (–)-enantiomer has a consid-

erably higher stability against oxidative degradation by human liver microsomes than the (+)-enantiomer or the racemate, which has so far been used as a drug.

Toxicity

The oral LD_{50} of metrifonate in male and female rats was 630 and 560 mg/kg, respectively (43), clearly demonstrating low acute toxicity. However, since Alzheimer drugs are intended for prolonged use, cumulative effects are of the highest importance and, considering the chemistry of this compound, genotoxicity from DNA alkylation has to be expected. This has indeed been observed in various test systems, such as *Salmonella thyphimurium* where *in vivo* (host-mediated) mutagenic activities of metrifonate on strain TA100 were fairly high, although it had low mutagenic activity *in vitro* and its administration resulted in urine of low mutagenic activity (44). Intraperitoneal administration of 0.48, 0.40 or 0.065 mmol/kg $^{14}CH_3$-labelled metrifonate to male mice resulted in maximum alkylation of liver and kidney DNA (evidenced by the formation of N-7 [^{14}C]methylguanine) 6 h after injection, and amounted to 6-8 and 0.8 μmol 7-MeG/mol guanine for the high and the low dose, respectively, corresponding to a covalent binding index of 4-5. The extent of methylation at guanine positions 0-6 was estimated to be around 0.002-0.01 μmol 0-6 MeG/mol guanine (45). In spleen and other intestinal organs, formation of potentially promutagenic DNA alkylation products proceeded at a much lower rate (46).

While the ability of metrifonate and dichlorvos to induce chromosome breakage was demonstrated in plant and Drosophila insect cells, this was not consistently observed in mammals. When mice were injected with acute doses of 10 mg/kg dichlorvos or 100 mg/kg metrifonate, no clastogenic effect was seen in bone marrow and testis cells. A 7-week treatment with 2 p.p.m. dichlorvos or 0.5 p.p.m. metrifonate for 5 days per week also failed to achieve such an effect. In an investigation of dominant lethal mutations, dichlorvos and metrifonate did not enhance the frequency of dead embryos but the frequency of pre-implantation losses was significantly increased in two specific periods of the seven investigated (47). In a human lymphocyte proliferation system exposed to 10-60 μg/ml of metrifonate for 72 h, sister chromatid exchanges were induced at all concentrations with the exception of the lowest one, but without a dose-response expression. A cell cycle progression delay was seen at 40, 50 and 60 μg/ml. In a mouse bone marrow system, 120 mg/kg (but not 30 or 60 mg/kg) induced exchanges but did not modify cell proliferation kinetics (48).

Although the offspring of pigs receiving four 50 mg/kg doses of metrifonate during pregnancy have been reported to show a high frequency of congenital tremor and cerebellar hypoplasia (49), a critical review of the literature failed to reveal conclusive evidence for a teratogenic or carcinogenic effect in higher vertebrates (50). Long-term follow-up of various large populations, both animal and human, that were subjected to mass chemotherapy for schistosomiasis has not revealed indications for cumulative toxicity.

In the mouse, metrifonate was found to be without apparent effect on the activity of microsomal monooxygenase enzymes (51).

Chronic administration at high doses inhibited spermatogenesis in rats and dogs (50). In a study of 18 Alzheimer patients treated with metrifonate for up to 7 months, no significant alterations were noted in erythrocyte, leukocyte or platelet characteristics or numbers that would suggest a deleterious effect of AChE inhibition on normal differentiation. Thus, any modification of developmental pathways appears to be compensated by other regulatory mechanisms in the intact organism (52).

Clinical Studies

Preliminary results of the first open trial of metrifonate in Alzheimer's disease were published in 1990 (53). In another clinical study conducted by the same group of researchers (54), 50 patients with probable AD completed a 3-month, double-blind study to compare metrifonate to placebo. Metrifonate was dosed to achieve a 40-60% inhibition of red blood cell AChE activity. The ADAS cognitive subscore (ADAS-Cog) served as the primary outcome measure. At the completion of 3 months of treatment, the metrifonate group score differed significantly from the placebo group score by 2.6 points ($p < 0.01$). A 0.75-point trend toward improvement occurred during treatment in the ADAS-Cog performance of the metrifonate group ($p = 0.15$), and a 1.10-point deterioration was found in the placebo group ($p < 0.02$). On the Global Improvement Scale (GIS), the two groups differed significantly on their changes from baseline to treatment phase ($p < 0.02$). Significant deterioration occurred in GIS scores ($p < 0.01$) and in Mini Mental State Examination (MMSE) scores ($p < 0.03$) in the placebo-treated group. Adverse effects were uncommon and did not require adjustment of the dose of metrifonate or discontinuation of treatment. A 52.3% mean decrease in red blood cell acetylcholinesterase activity was achieved. During up to 18 months of subsequent open metrifonate treatment of patients, a deterioration of 1.68 points per year in MMSE performance was found.

A third study (55), again by essentially the same research group, was designed to investigate sustained cognitive effects of metrifonate treatment in Alzheimer's disease. In an initial 1-month, patient-blind phase, all 47 participants were dosed to 50-70% blood cholinesterase inhibition. This was achieved by a loading dose of 2 mg/kg oral metrifonate daily for 5 days, followed by 0.95 mg/kg on day 6, and then by 2.9 mg/kg weekly. At the start of the subsequent 6-month, double-blind phase, patients were randomized to receive continued metrifonate treatment or placebo. All observed adverse events were mild and transient; 46 patients completed the study course. At endpoint, mean erythrocyte acetyl-

cholinesterase activities had recovered almost to prestudy levels for those switched to placebo. A statistically significant ($p < 0.03$) group difference was found in the ADAS-Cog, attributable to the fact that the metrifonate group had exactly maintained its mean baseline level of 20.6 points during the double-blind phase while the placebo group had declined 1.67 points ($p = 0.01$).

A large phase III, 6-month, parallel-design study, carried out at 25 clinical centers in the U.S., used daily dosing at lower levels and produced results that are much more in line with those obtained with other acetylcholinesterase inhibitors. The double-blind study enrolled 408 patients (age 45-90 yr) with mild to moderate AD who were randomized to either metrifonate (at a loading dose of 2 mg/kg/day followed by a maintenance dose of 0.64 mg/kg/day, corresponding to absolute daily doses of 30-60 mg for 24 weeks) or placebo. Patients with poorly controlled diabetes, asthma or chronic obstructive pulmonary disease, symptomatic benign prostatic hypertrophy, or a primary diagnosis of psychiatric disorders not related to Alzheimer's disease were not eligible. Eighty-eight percent of patients receiving metrifonate completed the study compared to 96% on placebo. Generally, the side effects of metrifonate were of mild intensity and transient, and were resolved during continued therapy. The most common side effects included diarrhea, leg cramps and rhinitis. There were no clinically relevant laboratory adverse events, including no liver function abnormalities and no appreciable effects on weight or blood pressure. The treatment difference was 2.85 ADAS-Cog points at 26 weeks ($p = 0.0001$) and the difference on the caregiver-related CBIC+ scale was also significant ($p = 0.0024$). The mean treatment effect for the total Neuropsychiatric Inventory (NPI) score at week 26 was 2.76 ($p = 0.016$). Statistically significant treatment differences were also observed for the mean change from baseline score as evaluated by the NPI in hallucinations ($p = 0.0002$), depression/dysphasia ($p = 0.026$), and apathy ($p = 0.034$). Additionally, although not of statistical significance, metrifonate tended to modify aberrant motor behaviors. Patients who completed the double-blind phase were eligible for inclusion in a 26-week, open-label phase, which is ongoing at the time of writing (56-58).

Bayer Corp. filed a New Drug Application (NDA) with the FDA on November 12, 1997, for metrifonate tablets with strengths of 50, 60 and 80 mg. Bayer AG's 1997 annual report (presented in March, 1998) contained an expression of confidence to the effect that metrifonate, along with other drugs, could be launched in 1999.

Manufacturer

Bayer AG (DE); Bayer Corp. (US).

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