DI - ISO PROPYLFLUOROPHOSPHONATE (DFP)

Its Pharmacology and its Therapeutic uses in Glaucoma and Myasthenia Gravis

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The alkyl fluorophosphonate group of compounds has attracted much attention recently, and its most active member, di-isopropyl fluorophosphonate, has now been the subject of some published work. The substance has the formula given below. It is a clear colourless liquid, purified by redistillation, slightly soluble in water, but readily soluble in organic solvents. Aqueous solutions are unstable and half hydrolysis occurs in 16 hours in the concentrations usually employed.

McCombie and Saunders (1946) described the preparation of the alkyl fluorophosphonates on both the laboratory and the technical scales with good yields. With the aid of the Cambridge Extra-mural School (Adrian *et al.*), these substances were shown to have a pronounced miotic action and could be lethal to animals in small doses. Dixon and Needham (1946) presented the biochemical aspect of the problem in a review of work done in the Chemical Warfare field. They indicated that the alkyl fluorophosphonates have a pronounced anti-choline esterase effect and that their most active members were able to inhibit the enzyme in concentrations as low as one in a hundred thousand million Molar. Comroe, Todd and Koelle (1946) reported the pharmacology of di-isopropyl fluorophosphonate (DFP) in man, showing 2 to 3 mgm. intramuscularly decreased the plasma and to a lesser extent the red cell choline esterase concentrations.

Mazur and Bodansky (1946) confirmed the

in vitro and in vivo inhibition of choline esterase activity by DFP. They showed that there was both a species and a tissue difference in the degree of inhibition after treatment with DFP. They studied its effect on choline esterase activity in blood plasma, red blood cells, muscle and brain tissue in vitro and correlated these findings with those in living animals, viz.:—the rabbit and the macaque following inhalation and intravenous injection of DFP. Man appears to be very sensitive to DFP and, in experiments involving the inhalation of DFP vapour, the choline esterase changes in human plasma and red blood cells largely bore out the impressions gained from animal work. With high concentrations of DFP there is a rapid onset of the inhibition of the enzyme. The regeneration of choline esterase activity was studied in the rabbit after the intravenous injection of 0.3 mg. of DFP and it was found that there was 100 per cent. restoration in the plasma in five days with 100 per cent. activity in the red blood cells in ten days while in brain tissue had only recovered to 90 per cent. after fifty days. It appears that DFP combines irreversibly with choline esterase for neither dialysis nor dilution will separate the enzyme-inhibitor mixture. Eserine on the other hand can be separated from choline esterase by both these procedures (Matthes, 1930, Straus and Goldstein, 1943). Hall and Ettinger (1937) showed that choline esterase activity had returned to normal after eserine intravenously in the dog within two hours. It has been suggested that the length of time required for the restoration of choline esterase activity after DFP is compatible with a resynthesis of the enzyme protein. Mazur (1946) gives some evidence for the existence in the body of an enzyme that

hydrolyses DFP when the latter is in high concentration in the organism.

Most of the information about DFP has been published in the U.S., but it is felt that the Americans would be the first to acknowledge that much of the original work in this field was done by teams in England who have, as yet, been unable to publish their work. For example, Mazur and Bodansky (1946) refer to earlier personal communications received from McCombie, Adrian, Kilby and Kilby (1941), who stated that the fluorophosphonates had a cholinergic effect similar to that of eserine and from Mackworth (1942), who showed that horse serum choline esterase when incubated with alkyl fluorophosphonates was inhibited. These are but two of many such references to be found in the American literature and the publication of the original British work can be anticipated.

From the therapeutic standpoint DFP opens up a wide range of fields but, so far, clinicians have only been able to apply the substance in two directions, viz.: that of glaucoma and that of myasthenia gravis. One difficulty has been that the aqueous solutions of DFP are unstable owing to hydrolysis taking place, and this has been overcome by the introduction of peanut oil (arachis oil) as a vehicle. Further, supplies of pure DFP are not readily available.

Leopold and Comroe (1946a) recorded the actions of DFP on the normal eye, expanding the earlier British work. They noted the prolonged miosis of up to three weeks' duration with a spasm of the ciliary muscle for three to seven days. There is usually a decrease in the intra-ocular tension, although occasionally there may be a transient rise before fall in pressure. The action outlasts any of the known miotics, and a 0.1 per cent. solution of DFP gives an action equal or greater than that of 1 per cent. eserine or 5 per cent. neostigmine bromide, and outlasts them both. After the removal of the ciliary ganglion, no miosis with DFP was observed as might have been expected from a drug inhibiting only the choline esterase and also showing that DFP had no direct effect on the iris musculature. Anderson (1905) showed there was an absence of response of the iris to eserine after the removal of the ciliary ganglion.

Pupil dilations effected by atropine have been easily overcome by ocular administration of DFP. Fluorescein appears to penetrate the blood-aqueous humour membrane faster, while the permeability to inulin was unaltered after DFP. Further experiments on the isolated cat's head along the lines adopted by Davson and Quilliam (1940) and Duke-Elder, Quilliam and Davson (1940) would be helpful in a more exact study of the permeability changes in this region following DFP.

Leopold and Comroe (1946b) have studied the use of 0.05 per cent., 0.1 per cent. and 0.2 per cent. DFP in peanut oil in glaucoma, and found that cases unrelieved by eserine responded readily to 0.1 per cent. DFP instilled into the conjunctival sac. However, the action of DFP in the glaucomatous eye lasts for about 12 hours or so compared with an action of as many days in the normal eye. Daily instillation of 0.1 per cent. DFP drops in an oily base under hospital conditions, and with control of the case by careful tonometry was recommended and should be insisted upon until a wider experience of the action of the drug is obtained. The short-lived action of DFP in cases of raised intra-ocular tension lends some evidence to the interesting suggestion that there may be an upset of the acetyl choline-choline esterase mechanism in glaucoma. There are certain side effects such as ciliary spasm and brow ache which may prove troublesome in ophthalmic therapy.

Wilson (1946) studied the effect of DFP in myasthenia gravis and found that on prolonged administration in one case there was a marked diminution of the prostigmine requirements (a fall from 2 mg. to 0.2 mg. after three weeks of daily DFP). Another case was able to perform muscular movements without undue fatigue after a course of DFP alone.

Mendel and Hawkins (1946) have shown that DFP inhibits, somewhat more readily than choline esterase, another enzyme system, that of the pseudo choline esterases. If effects on the choline esterase enzyme system were required it would seem that relatively high and prolonged dosage would be required. This may well explain the divergent results of Wilson in his study of myasthenia gravis.

The symptoms produced by DFP (2 to 3 mg. intramuscularly) in man have been carefully studied by Comroe, Todd and Koelle (1946). Gastro-intestinal disturbances are most common and the symptoms, in order of frequency of occurrence, are:—nausea, epigastric "discomfort," indigestion or belching, anorexia and occasionally diarrhoea, vomiting or abdominal cramps. There were no marked changes in cases exhibiting these symptoms on fluoroscopic examination. Less frequently, patients complained of symptoms referable to the central nervous system, viz.:—dizziness, "shakiness," weakness and frequent dreams or nightmares. No increase in sweating or salivation was observed, and in two female cases there was a slight dribbling of urine probably arising from a parasympathetic stimulation effect. Prolonged administration may give rise to any of the above symptoms and in addition headache may occur but no definite miosis was seen.

Compared with neostigmine, DFP produced side effects more commonly and these arose in spite of administration of atropine (1/100 gr.) in an attempt to control the gut symptoms. There were no changes in liver, kidney or haemopoietic function ascribed to DFP. No significant change occurred in the cardiovascular system and no bronch-constriction was observed but asthma was considered a contraindication to the use of DFP. It can be thus seen that DFP in therapeutic dosage is a safe medicament.

Experimental work has shown that DFP may increase the sensitivity of the blood pressure to in-

flexions due to acetyl choline (Modell, Krop, Hitchcock and Ricker, 1946). The isolated rabbit heart preparation may be used to demonstrate that DFP can markedly potentiate the effect of small doses of acetyl choline and also increase the sensitivity of the isolated organ (Quilliam and Strong, unpublished observation). The isolated frog rectus muscle when treated with DFP exhibits an increase of sensitivity to acetyl choline of the same order as that effected by eserine and as with the heart the change is permanent, being unaffected by repeated washings. A convenient and powerful anti-choline esterase is thus available for experimental work (Quilliam, unpublished observations).

This review may serve to show how a substance first extensively studied in wartime research has now shown its peacetime potentialities. If the promise of its usefulness is amply fulfilled, DFP may well take its place alongside the sulphonamides and penicillin as one of the advances of the twentieth century.

Summary

The pharmacology of di-iso propylfluorophosphonate (DFP) is reviewed, and its action as a powerful anti-choline esterase is indicated. The therapeutic role of DFP as an agent in the reduction of intra-ocular tension in glaucoma and as a potentially useful weapon in myasthenia gravis are described.

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