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INHIBITION OF TUBULIN ASSEMBLY BY ANTILEPROSY DRUG DAPSONE

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The effect of dapsone on assembly-disassembly process of bovine brain tubulin was examined. The drug was found to readily bind tubulin dimer and that in its presence colchicine binding to tubulin was enhanced. Although dapsone associated with tubulin at a site other than the colchicine binding site, distinct inhibition of microtubule assembly was detected.

It has recently been shown that several drugs which affect the central nervous system impair microtubule function (1,2,3). However, it is not known whether those drugs which preferentially localize in the peripheral nerves affect tubulin function. Dapsone (4-4' diamino diphenyl sulfone) which is widely used for the treatment of leprosy, tuberculosis and various infectious diseases is shown to be localised in the peripheral nerves in detectable amounts (4). It has also been observed that regular intake of dapsone for several years causes adverse effects such as dermatitis, neuritis and deformities in many leprosy patients (5). Neurotoxic effect of dapsone on healthy individuals has been recorded (6). That dapsone and other sulfones penetrate to the sciatic nerves of dog and sheep is also known (7,8). In this communication we have described binding of dapsone with tubulin, its effect on binding of colchicine to tubulin and on the assembly-disassembly process of microtubules.

MATERIALS AND METHODS:

The following chemicals were obtained from Sigma Chemical Company. 2-(N-Morpholino ethane) sulfonic acid (M-8250), ethylene glycol bis-(β-amino ethyl ether) N,N'-tetra acetic acid (E-4378), guanosine 5' triphosphate (G-5631), Tris (T-1503), and glycine (G-7126). Colchicine and Dapsone were purchased from John Baker Inc. Colarado U.S.A. [^{3}H]Colchicine was purchased from New England Nuclear Company. [^{3}H]labelling of dapsone was done by the Isotope Division of Bhabha Atomic Research Centre.

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Bovine brains were obtained from the Government abattoir immediately after slaughter. Tubulin was purified by Weisenberg's method (9) modified by Bhattacharya et al (10). Pure protein was concentrated and stored as described earlier (11). Protein was estimated by Lowry's method (12) taking bovine serum albumin as the standard. Homogeneity of tubulin was checked by urea gel electrophoresis (13). The gels and samples were prepared as explained before (11). Percent purity of the protein was calculated from densitometric scanning and tubulin was found to be 90% pure.

In vitro assembly and disassembly of tubulin was observed in buffer pH 6.5 containing 100 mM Morpholino ethane sulphonic acid, 0.5 mM $MgCl_2$, 1 mM GTP, 1 mM ethylene glycol bis (β-aminoethyl ether) N,N'-tetra acetic acid and 4M glycerol. Turbidity was measured at 350 nm using a Gilford 2000 spectrophotometer. Colchicine and dapsone binding was studied by standard filter assay method using Whatman Diethyl aminoethyl cellulose DE 81 filter discs (9) and counted as mentioned earlier (11).

Dapsone solution upto 5×10^{-4}M were made by diluting the concentrated solution in methanol with aqueous buffer. The control in each case contained the amount of methanol contained in experimental solution.

RESULTS AND DISCUSSION:

Dapsone binding examined under those conditions in which colchicine binds to tubulin optimally indicated that this drug readily bound to the tubulin dimer (Fig. 1). Due to the insolubility of dapsone in aqueous buffer higher concentrations of the drug could not be examined for reaching the saturation level. But from this graph it is evident that when 5×10^{-4}M dapsone is used 3 to 4 moles of the drug are found to bind one mole of

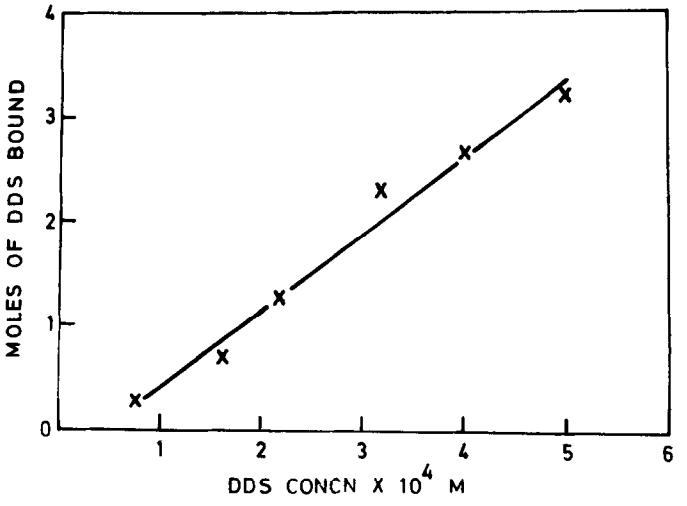
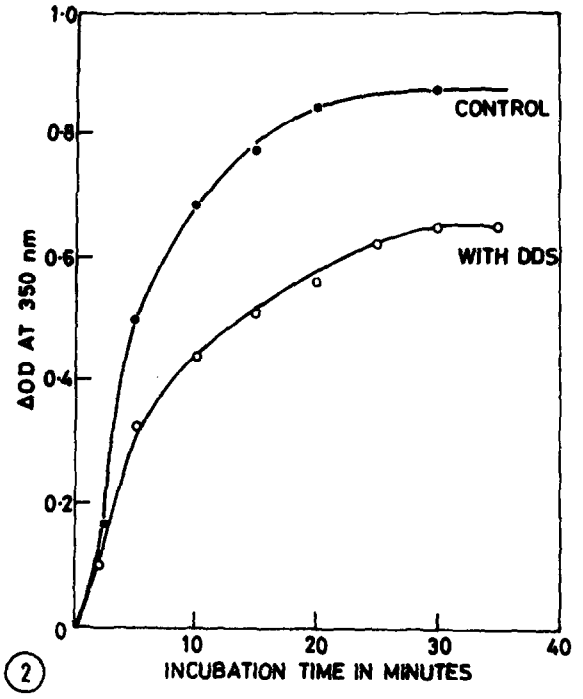


Figure 1. Dapsone binding to tubulin at 37°C in phosphate buffer 6.5 containing 0.1 mM GTP and 0.1 mM Mg^{++}.



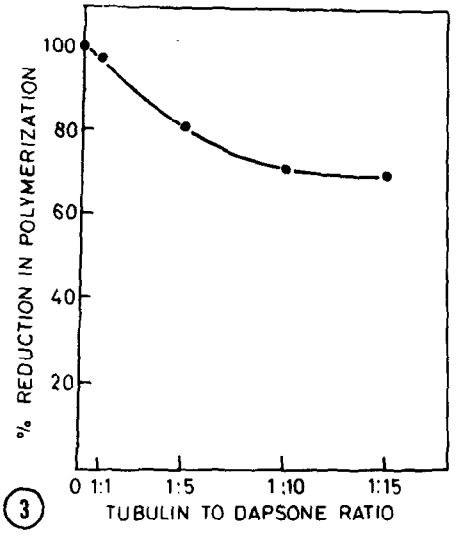


Figure 2. Microtubule polymerization in presence of dapsone. Tubulin 2.5 x 10^{-5}M; dapsone 2.5 x 10^{-4}M in MES buffer pH 6.5 (0.1 M MES, 1 mM EGTA, 1 mM GTP and 0.05 mM Mg^{++}).

Figure 3. Percent reduction in polymerization in the presence of different concentrations of dapsone. Tubulin 2.5 x 10^{-5}M.

tubulin dimer and there appears to be room for binding of more drug. Similar observations have been made with chlorpromazine binding to tubulin in which case 8 to 9 moles were shown to bind per mole of tubulin (14) at the saturation level.

Since dapsone associated with tubulin, its effect on the assembly-disassembly process was examined. The assembly profile of tubulin in the presence of dapsone in 1:10 protein-drug ratio showed (Fig. 2) 27% inhibition of microtubule formation. Lower drug concentrations (1:1) did not show significant effect (Fig. 3). Higher concentration (1:15) did not cause enhanced inhibition of microtubule over what was seen at ten fold. The observed lack of progressive inhibition with increased drug concentration could be due to the non-availability of the tubulin dimers for the drug to bind with and block polymerization. This is more likely because dimers are required for polymerization and drug binding, both dependent on time course.

Since polymerization process is probably faster than the drug association it would lead to unavailability of dimers for drug binding under these conditions.

This appeared to be the case when we compared the duration for completion of polymerization. In the presence and absence of the drug, microtubule assembly was found to be complete within 20 minutes. Lag period was also the same in both the systems. This data showed that in the presence of drug, only drug-free tubulin dimers participate in the polymerization. Other drugs pentobarbital (1) chlorpromazine and trifluoperazine have been shown to inhibit tubulin assembly, whereas enhancing effect on polymerization has been recorded in the case of promethacine (3).

The result of disassembly of tubulin at 0°C is shown in Fig. 4. Tubulin polymerized in the presence of dapsone, depolymerized to the same extent as the tubulin which was polymerized in the absence of the drug. This can happen if the polymerized tubulin contains only drug free dimers as was indicated by the polymerization experiments carried out in presence of various concentrations of the drug. These observations further confirm the earlier conclusions that the tubulin dimers which participated in the

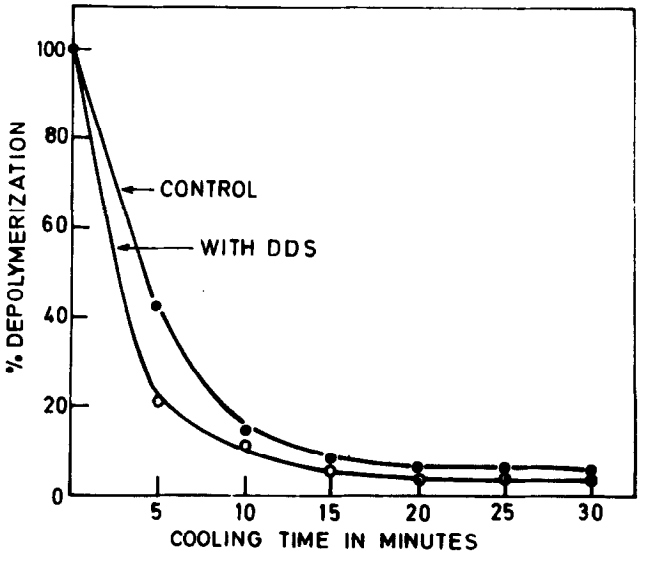


Figure 4. Percent depolymerization at 0°C. Tubulin 2.5 x 10^{-5}M, dapsone 2.5 x 10^{-4}M in polymerizing buffer.

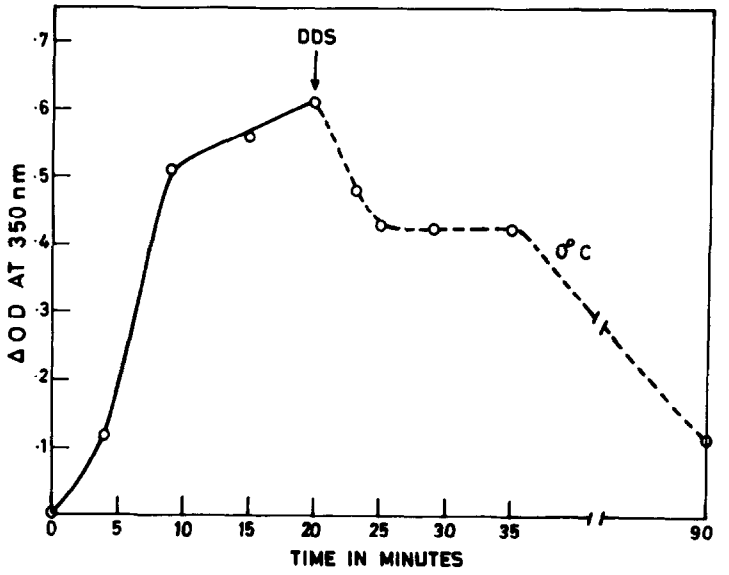


Figure 5. Effect of dapsone on polymerized microtubules. Tubulin 2.5×10^{-5}M, dapsone. 2.5×10^{-4}M in polymerizing buffer.

microtubule formation were probably those which were not complexed with the drug. It, therefore, seemed that although drug bound to the α, β dimer, this binding affected only the assembly process, but had no effect on disassembly process. In order to check further, whether drug had any affinity to the polymerized tubulin dapsone was added 20 minutes after the assembly process was initiated and had reached its maximum level (Fig. 5). The data showed that addition of dapsone at 37°C caused immediate disassembly which reached a steady state after 5 minutes. However, further disassembly occured only at 0°C. These data can be explained by the treadmilling theory of Margolis (15) according to which at the steady state there is a precise balance of net unit gain at the Assembly end and subunit loss at the Disassembly end of the microtubules in solution. When the drug was added to the solution, free subunits bound to the drug and the equilibrium shifted towards the disassembly side and reached the steady state after 5 minutes. Further disassembly occurred only after the temperature was brought to 0°C. Tubulin-dapsone complex did not participate in the polymerization process, because drug binding probably modified the conformation of the dimer. These assumptions were confirmed when it was found that dapsone binding leads to

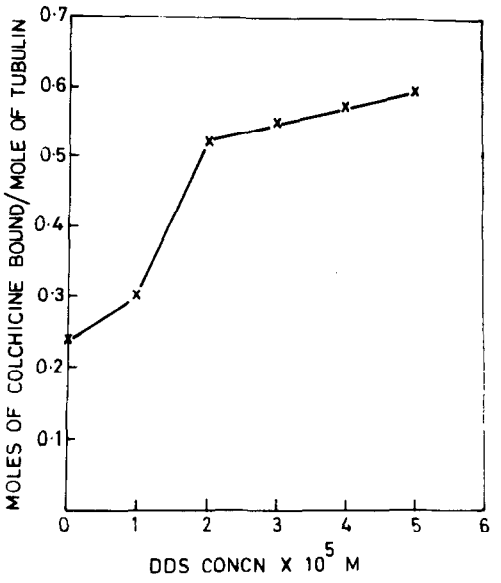


Figure 6. Colchicine binding to tubulin in presence of dapsone. Tubulin 10^{-5}M, colchicine 10^{-5}M.

the enhanced binding of colchicine (Fig. 6). Increased colchicine binding could only occur when dapsone binds at a site other than the colchicine binding site and by occupying the other site somehow modifies the colchicine binding site resulting in enhanced colchicine association as noted in other systems such as vinca alkaloids (16). We have earlier shown that colchicine binding site is involved in the polymerization process (11). Therefore, if the site was modified as a result of dapsone association it was rendered incompetent to participate in the polymerization process.

Thus it is evident that dapsone modified the colchicine binding site making the tubulin dimer incapable for polymerization. These findings have two important implications: 1. Since dapsone binds nerves readily (8), it would become unavailable in the free form for efficacious action in vivo for bacteriostatic function. Although, indirectly, this indicates how bacterial resistance is likely to develop due to lack of free drug availability at susceptible tissue sites. 2. Dapsone binding to the tubulin dimer alters the protein conformation making it incompetent for participating in its normal function that is microtubule formation. The modified tubulin is

likely to elicit immune response *in vivo*, thus contributing to nerve damage giving rise to neuritis. Since tubulin is one of the major protein components of the axons and dendrites of neurons its modification by dapsone would explain the cause of adverse reactions observed during long term dapsone therapy in the case of leprosy patients.

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