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ONCODAZOLE (R 17934) AN INHIBITOR OF THE TURNOVER OF PHOSPHATIDYL INOSITOL IN CONCANAVALIN A INDUCED LYMPHOCYTES

JULIAN J. MILLER

K.R.U.F. Institute of Renal Disease, Cardiff Royal Infirmary, Cardiff, South Wales, U.K.

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Abstract—The benzimidazole derivative, oncodazole, has known colchicine-like activity toward the micro tubular apparatus. Our investigations have now shown that this similarity is extended to the effects of oncodazole on the turnover of ^{32}p labelled phosphatidyl inositol in lymphocytes stimulated with Concanavalin A. This inhibition was dose dependent, at 10^{-3}M oncodazole, there was inhibition of both basal and Con-A stimulated ^{32}p incorporation, whilst at 10^{-5}M there was no basal inhibition and there was increased ^{32}p incorporation into phosphatidyl inositol with Con-A.

There is now increasing evidence to suggest that the imidazole class of drugs, including the benzimidazoles, may have some previously unforseen effects on the immune system. It is known for example that niridazole (Ambilhar) is a potent inhibitor of cell mediated immunity [1, 2] and will prolong the survival of transplanted allografts. [3] Miconazole (Daktarin) has recently been shown to inhibit mitogen induced lymphocyte blast transformation [4]; metronidazole (Flagyl) has been shown to prolong allograft survival [5], as has dacarbazine (DTIC) [6]. These cited examples are suppressors of the immune system, but there are some imidazoles which are known to stimulate; these include levamisole [7] and thiabendazole [8].

During investigations into the properties of imidazoles our attention was drawn to the properties of oncodazole (R 17934), methyl [5-(2-thienylcarbonyl)-IH-benzimidazol-2-yl] carbamate, a synthetic compound exhibiting antitumoural activity in both experimental and human neoplasms [9] and causing complete disappearance of microtubules from mitotic and interphase cells in culture [10]. This antimitotic property of oncodazole has been said to be indentical in its effect on the microtubular apparatus to colchicine [9]. It has recently been shown that colchicine affects many membrane properties, for example, lateral mobility of surface membrane markers and receptors; many of these events involve concanavalin A (Con A) including the mitogen induced aggregation of PMN's, fibroblasts and hepatoma cells which are inhibited by colchicine [11–13] and the reversal by colchicine of Con A inhibitors of lymphocyte immunoglobulin receptor capping [14–16]. In addition to these effects on membranes, colchicine has also been shown to inhibit Con A induced lymphocyte transformation early on in blast transformation [17, 18] and it has also been shown to inhibit phosphatidyl inositol (P I) turnover in lymphocytes [19] induced by Con A. It will inhibit both basal and Con A stimulated incorporation of [^{3}H] inositol into phosphatidylinositol.

These various findings led us to investigate whether oncodazole, as an inhibitor of microtubular function, would likewise inhibit the turnover of membrane phosphatidyl inositol in both the resting and stimulated lymphocyte. The results are shown in Table 1; the term '^{12}P ratio' refers to the ratio of the test situation: control for each individual experiment. At a concentration of 10^{-3}M the drug inhibited basal turnover of ^{32}P since in all cases the c.p.m. were below those of the control with a mean ^{32}P ratio of 0.84. A similar mean ration of 0.83 was obtained in the presence of Con A, indicating that oncodazole also inhibited Con A stimulated incorporation of ^{32}P. At a concentration of 10^{-5}M oncodazole there was little difference between the controls and those lymphocytes treated with this concentration of drug. This suggests that there was no inhibition of basal turnover. In the presence of Con A and oncodazole (10^{-5}M) there was definite stimulation of the lymphocytes with a mean ^{32}P ratio of 1.52, being no different from the Con A stimulated cells alone. In other experiments (data not included) 10^{-4} M oncodazole gave supporting evidence that this drug inhibits both unstimulated and Con A-stimulated ^{32}P incorporation into PI in a dose-dependent manner.

From the work of Schellenberg and Gillespie, using [^{3}H] inositol incorporation into phosphatidyl inositol of lymphocyte membranes, colchicine exerts its effects through a direct interaction with tubulin or tubulin-like protein in membrane. Oncodazole possesses similar properties in its inhibition of tubulin polymerization [20] and may this act by a similar mechanism. It is, however, possible that oncodazole may directly affect an enzyme or enzymes involved in lymphocyte membrane PI turnover.

It is now well established that the induction of lymphocyte blast transformation by lectins, including Con A, results in a rapid triggering in the turnover of membrane phosphatidyl inositol [21]. It is believed that this enhanced PI turnover is an essential, if not the primary, requisite of lymphocyte activation. Furthermore, these studies have shown that inhibition of PI turnover with oncodazole inhibits mitogen induced blast transformation. The implications of these findings are yet to be elucidated but it seems plausible that if closely related analogues of oncodazole show the same effect toward PI turnover in activated lymphocytes then

Table 1. Effects of Concanavalin A and oncodazole on ^{32}p incorporation into PI

	Experiment number														
	1		2		3		4		5		6		7		
	c.p.m.	^{32}p ratio	c.p.m.	^{32}p ratio	c.p.m.	^{32}p ratio	c.p.m.	^{32}p ratio	c.p.m.	^{32}p ratio	c.p.m.	^{32}p ratio	c.p.m.	^{32}p ratio	^{32}p ratio
Control	939	—	2217	—	5192	—	394	—	282	—	666	—	2377	—	—
Con A stimulated	1366	1.45	2997	1.35	7056	1.36	627	1.59	796	2.82	1117	1.67	3313	1.39	1.66
Oncodazole, 10^{-3} M	853	0.91	1820	0.82	4896	0.74	—	—	—	—	462	0.69	—	—	0.84
Oncodazole, 10^{-3} M + Con A	974	1.04	2018	0.91	3686	0.71	—	—	—	—	461	0.69	—	—	0.83
Oncodazole, 10^{-5} M	1023	1.09	1534	0.69	—	—	440	1.11	348	1.23	—	—	2117	0.89	1.04
Oncodazole, 10^{-5} M + Con A	1002	1.07	2173	0.98	—	—	795	2.01	680	2.41	—	—	2676	1.13	1.52

One to two × 10^7 guinea pig lymphocytes isolated by density gradient centrifugation were incubated in duplicate in 1 ml of phosphate free HEPES–Ringer, pH 7.4, supplemented with 11 mM glucose together with 10 μCi ^{32}p for 60 min at 37°. Con A (40 μg) and oncodazola, with or without Con A, were added and incubated for a further 60 min. The incubation was stopped by the addition of 3.75 ml chloroform–methanol (1:2, v/v), mixed vigorously, and allowed to stand for 20 min followed by the addition and further mixing of 1.25 ml chloroform and 1.25 ml KCl (2 M).

Following centrifugation the lower phase was removed and evaporated to dryness. The extract was redissolved in chloroform (100 μl) and chromatographed on Silican gel G plate using chloroform–methanol–acetic acid–H_2O (25:15:4:2, by vol) as solvent. The PI was localised by I_2 vapour, removed from the t.l.c. plate and the ^{32}p content determined in a liquid scintillation counter.

it is possible that these compounds may have some role to play in the suppression of lymphocyte activity toward other blast inducing agents.

These findings contrast sharply to the known activity of the immunosuppressive agent imuran, which does not appear to have a single mode of action. There have been several reports as to the mode of action of this imidazole: interference with coenzymes, incorporation into nucleic acids, inhibition of enzymes, alteration of purine interconversions, inhibition of *de novo* purine synthesis and binding to amino acids. [22]. However, there does not appear to be any evidence that this immunosuppressant affects PI turnover in activated lymphocytes as does oncodazole.

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