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Effects of Doxorubicin on Maturation of Human Monocytes in Adherent and Non-adherent Cultures

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Purified human monocytes were cultured for 2 h, 88 h, and 10 days in plastic tubes (adherent) and for 10 days in Teflon foil bags (non-adherent). Monocytes were incubated with doxorubicin by two short-term exposures (750 or 1500 ng/ml) for 1 h or by continuous exposure (75 ng/ml). Maturation was monitored by measuring the intracellular activity of three metabolic enzymes and two acid hydrolases. Expression of receptors for the Fc moiety of immunoglobulin G (FcRI, FcRII, FcRIII), CD14, and HLA-DR was assayed by indirect immunofluorescence with monoclonal antibodies. In the presence of doxorubicin, the adherent capacity, the yield, and the enzyme activities reflecting growth and intermediary metabolism were similar to the control groups. However, doxorubicin reduced the expression of FcRI (32–45%), FcRII (10–26%), CD14 (20–37%), and HLA-DR (25–34%) on the monocyte-derived macrophages. Expression of FcRIII was not detectable after 10 days of culture.
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INTRODUCTION

MONOCYTES AND MACROPHAGES, part of the mononuclear phagocyte system, are a first-line defense against the development and spread of tumors [1, 2]. Doxorubicin is active against a wide range of tumors, including leukemia, lymphoma, sarcoma, and breast, small cell lung, and ovarian cancers [3, 4]. In view of the limited progress so far with the use of cytostatic agents in metastatic disease, non-specific stimulation of the immune system by biological response modifiers has been studied [5]. Cytotoxicity was synergistic when doxorubicin was combined with interferon [6, 7]. However, cancer chemotherapy can both immunosuppress and activate the mononuclear phagocyte system [8].

We have investigated *in vitro* the effects of doxorubicin on the maturation and differentiation of human monocytes. We assayed five enzyme activities as indicators of monocyte maturation: glucose-6-phosphate dehydrogenase (G-6-PDH), phosphohexose isomerase (PHI), isocitrate dehydrogenase (ICDH), acid phosphatase (ACP), and *N*-acetyl-β-glucosaminidase (NAG). We also examined the expression of three distinct classes of receptors for the Fc moiety of immunoglobulin G [FcRI (CD64), FcRII (CDW32) and FcRIII (CD16)] which have been identified on human mononuclear phagocytes [9, 10] and allow killing of antibody-coated target cells [11–13]. We also studied gp55 (CD14), a membrane protein which is expressed in abundance on the surface of mature monocytes and macrophages [14, 15], and HLA-DR which is expressed on antigen-presenting cells [16].

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MATERIALS AND METHODS

Peripheral blood monocytes

Monocytes were purified from mononuclear cells of healthy volunteers with counterflow centrifugation monitored by continuous flow cytometry [17]. Monocyte fractions were over 95% pure and viability was more than 98%.

Monocyte cell cultures

Freshly isolated monocytes were cultured at 3×10^5/ml in RPMI 1640 (Dutch modification), with 20 mmol/l HEPES buffer (Flow) supplemented with freshly added 2 mmol/l L-glutamine (Gibco), 1 mmol/l pyruvic acid (Sigma), 40 μg/ml gentamicin (Boehringer) and 10% heat-inactivated pooled human serum in a humidified incubator with 5% CO_2 in air at 37°C. For adherent culturing 1 ml of the cell suspension was seeded in plastic flat-bottomed tubes (Costar 3393, 16 × 93 mm; Cambridge) and harvested at different times (2 h, 88 h, and 10 days). Cultures were done in duplicate. The supernatant and non-adherent cells were decanted, and the loosely adherent cells removed by rinsing six times with 1 ml phosphate buffered saline/1% bovine serum albumin (PBS/BSA) at 37°C. By addition of 0.5 ml mmol/l EDTA/0.1% BSA the adherent cells were lysed to release their enzyme content. The lysates were stored at −80°C for later measurement of enzyme activity and DNA content. Teflon foil bags (Dupont de Nemours) were used for culturing 10 ml non-adherent monocyte suspensions [18]. After 10 days cells were recovered by needle aspiration after cooling the bags to 4°C for 1 h and gentle kneading. The cells were washed and resuspended in PBS. Samples were taken for enzyme measurements (10^6 cells), and stored at −80°C after pelleting and lysing in 1 ml EDTA/BSA.

Doxorubicin exposure

Doxorubicin was purchased from Laboratoire Roger Bellon (Neuilly sur Seine, France) and diluted in sterile saline. Two schedules were used: continuous exposure for 10 days and a short-term exposure for 1 h. For continuous exposure, monocytes were added directly to culture medium containing doxorubicin 1–1000 ng/ml. For the short-term exposure, 10^6 monocytes per ml were incubated with doxorubicin 10–10,000 ng/ml. Culture without doxorubicin served as control. Incubation tubes were cooled on iced water, the cells were collected with a rubber policeman and washed four times with culture medium before seeding them into the plastic tubes or Teflon foil bags. After culture for 10 days we measured the doxorubicin and doxorubicinol concentrations in the culture supernatants of the cells grown in Teflon foil bags by high-pressure liquid chromatography [19].

Flow cytometry

Flow cytometry was done on a System-50H Cytofluorograf (Ortho Instruments, Westwood, Massachusetts), and the data were accumulated in a multichannel analyzer (Nuclear Data, ND 100). Because doxorubicin autofluoresces it is possible to measure doxorubicin assimilation by monocytes and macrophages. We measured uptake of this drug in short-term incubated monocytes before and after 10 days' culture, and in cells co-cultured with doxorubicin for 10 days. Binding of specific monoclonal antibodies (mAbs) to FcRI, FcRII, FcRIII, CD14, and HLA-DR on doxorubicin-treated cells was assayed by indirect immunofluorescence (IF) with: 197 (anti-FcRI; Medarex, West Lebanon, New Hampshire), IV.3 (anti-FcRII; Medarex), anti-leu 11b (anti-FcRIII; Becton Dickinson), WT14 (which reacts strongly with human monocytes and macrophages and belongs to the CD14 cluster of differentiation) [15], and OK-DR (anti HLA-DR; Ortho). After culture in Teflon foil bags monocytes were harvested, washed and suspended (3×10^6/ml) in IF buffer (PBS/BSA 1.0% containing 0.1% sodium azide). 100 μl cell suspension was incubated with an equal volume of saturation levels of mAb for 30 min at 4°C. Cells binding the mAb were detected with goat anti-mouse Ig (heavy and light chain) conjugated to fluorescein isothiocyanate (Cappel, Malvern, Pennsylvania), diluted 1:50 in IF buffer. After 30 min at 4°C in the dark, the cells were washed twice and fixed with 1% paraformaldehyde for analysis. The fluorescence intensity from 5000 cells was measured. For all analyses, the gatings (red forward and right angle scatter) were set around the macrophage population and the mean fluorescence intensity (expressed in arbitrary fluorescence units/cells) was calculated from the histograms [20].

Enzyme and DNA measurements

G-6-PDH, PHI, and ICDH were assayed by fluorimetric measurement of NADPH generated from NADP in the presence of appropriate substrates with either direct or coupled systems; NAG and ACP were measured by fluorimetric assay of 4-methylumbelliferone released from linked derivatives [21]. Preliminary experiments verified the linearity of the system with respect to incubation time and enzyme activity. After subtraction of appropriate blanks (2 mmol/l EDTA/0.1% BSA), activity was expressed in nmol/min per μg DNA. The DNA content of the lysate ws measured by fluorescence of the complex with 4,6-diamidino-2-phenylindole-2-HCl [22].

RESULTS

Intracellular doxorubicin in non-adherent monocytes

After both short-term exposure to 10–10,000 ng/ml and in cells continuously exposed for 10 days to lower doses (1–1000 ng/ml) cellular fluorescence was linearly correlated with the concentration of doxorubicin. The mean fluorescence of the short-term exposed monocytes, before and after culturing, was similar to that of the cells which were continuously cultured (data not shown).

Extracellular doxorubicin in supernatants of non-adherent monocytes

No drug metabolites were found in the supernatants when monocytes were continuously exposed to doxorubicin at 1–250 ng/ml. However, at 500–1000 ng/ml, we measured doxorubicinol concentrations of 15.6–32.1 ng/ml [i.e. 3.2% (S.D. 0.1)] of the originally added doxorubicin concentration. The residual amount of parent compound after 10 days of continuous exposure to 1–1000 ng/ml was, for all measured points, still 24% (2) of the original quantity. In the short-term exposures to 10–10, 000 ng/ml, no derivatives were found, and the doxorubicin concentrations in the culture supernatants were always less than 0.25% of the original.

Doxorubicin and cell recovery

When doxorubicin was used at concentrations above 200 ng/ml in continuous exposure, and above 2000 ng/ml in the short term, cell recovery was halved (Table 1) and most (over 70%) of the harvested cells were dead. The monocytes had not matured into large macrophages but remained small with vacuoles. For further studies we used doxorubicin concentrations that were comparable to those clinically, i.e. 75 ng/ml for continuous exposure, and 750 or 1500 ng/ml for the short term.

Table 1. *Percentage cell recovery after doxorubicin exposure in adherent and non-adherent cell cultures*

	Adherent			Non-adherent
	2 h	88 h	10 days	10 days
Control	19.2 (3.9)*	27.7 (5.1)	31.8 (12.1)	83.6 (5.0)
Short-term 750 ng/ml	20.5 (2.7)	28.5 (4.3)	30.7 (9.4)	84.4 (6.3)
Short-term 1500 ng/ml	17.1 (6.2)	23.9 (2.2)	24.0 (5.5)	86.9 (7.0)
Continuous 75 ng/ml	17.4 (3.3)	22.9 (2.6)	23.9 (8.2)	78.1 (9.0)

*Mean (S.D.), $n = 6$.

Recovery = DNA content in lysate/content in 1 ml lysed uncultured monocytes for adherent, and harvested cells/cells initially seeded for non-adherent.

Table 2. Effects of doxorubicin on enzyme activities in adherent and non-adherent cultured monocytes

Enzyme	Control	Doxorubicin (ng/ml) Short-term 750	Short-term 1000	Continuous 75	Total
G-6 PDH					
Adherent					
2 h	4.47 (0.56)*	4.65 (0.86)	4.94 (0.81)	5.21 (0.80)	4.80 (0.82)
88 h	41.1 (8.5)	39.6 (14.1)	38.6 (15.8)	35.1 (10.6)	38.3 (13.2)
10 days	112 (23)	113 (23)	116 (21)	104 (30)	112 (25)
Non-adherent					
0 h	1.38 (0.32)				1.38 (0.32)
1 h	3.14 (0.90)	4.08 (1.70)	3.36 (0.76)	3.41 (0.99)	3.51 (1.14)
10 days	83.3 (16.5)	79.1 (12.6)	74.9 (12.5)	79.8 (13.3)	79.2 (12.4)
PHI					
Adherent					
2 h	25.5 (3.1)	28.1 (5.0)	27.9 (10.9)	33.9 (3.5)	28.9 (6.8)
88 h	62.8 (18.1)	56.8 (19.5)	61.5 (22.8)	59.2 (17.6)	60.1 (19.7)
10 days	157 (61)	148 (49)	162 (52)	156 (65)	156 (57)
Non-adherent					
0 h	8.73 (3.95)				8.73 (3.95)
1 h	31.1 (9.9)	42.8 (18.3)	35.9 (6.2)	36.4 (9.6)	36.9 (11.4)
10 days	154 (27)	147 (25)	137 (22)	142 (25)	143 (22)
ICDH					
Adherent					
2 h	2.27 (0.35)	2.22 (0.17)	2.73 (0.43)	2.99 (0.95)	2.53 (0.64)
88 h	4.95 (0.85)	4.51 (1.30)	4.61 (1.24)	5.24 (1.14)	4.83 (1.19)
10 days	9.46 (2.91)	8.52 (2.28)	9.18 (1.81)	9.03 (2.66)	9.05 (2.47)
Non-adherent					
0 h	0.30 (0.04)				0.30 (0.04)
1 h	0.93 (0.45)	1.32 (0.89)	0.91 (0.69)	1.12 (0.58)	1.08 (0.60)
10 days	7.21 (1.74)	6.94 (1.79)	6.27 (1.49)	6.31 (1.75)	6.69 (1.51)
ACP					
Adherent					
2 h	0.22 (0.02)	0.22 (0.03)	0.22 (0.04)	0.23 (0.03)	0.22 (0.03)
88 h	3.04 (1.03)	3.17 (1.16)	2.90 (0.83)	2.84 (0.75)	2.99 (0.97)
10 days	7.23 (2.78)	6.92 (2.06)	6.93 (1.75)	6.01 (1.61)	6.77 (2.15)
Non-adherent					
0 h	0.18 (0.03)				0.18 (0.03)
1 h	0.29 (0.03)	0.35 (0.07)	0.37 (0.05)	0.31 (0.03)	0.33 (0.05)
10 days	5.50 (1.11)	5.73 (1.37)	5.75 (1.96)	5.48 (1.52)	5.61 (1.32)
NAG					
Adherent					
2 h	1.20 (0.35)	1.12 (0.31)	1.18 (0.40)	1.32 (0.56)	1.20 (0.42)
88 h	3.63 (1.09)	3.52 (1.26)	3.59 (1.60)	3.51 (0.91)	3.56 (1.24)
10 days	11.6 (2.6)	12.5 (1.8)	14.2 (2.1)	14.6 (3.2)	13.2 (2.8)
Non-adherent					
0 h	0.60 (0.11)				0.60 (0.11)
1 h	0.78 (0.05)	0.98 (0.21)	0.94 (0.13)	0.79 (0.05)	0.87 (0.14)
10 days	21.7 (7.9)	23.9 (9.2)	26.5 (11.1)	23.1 (11.6)	24.0 (8.9)

*Mean (S.D.), $n = 6$.

Enzyme activities were measured in the lysates of adherent cell cultures after 2 h, 88 h, and 10 days. Non-adherent cells were assayed for enzyme activity by measuring lysates of cryopreserved monocytes, 1 h incubated monocytes, and day 10 harvested cells. Activities are expressed in nmol/min per μg DNA.

Compared with control cells, no significant changes were seen in adherence capacity of the plastic-cultured monocytes after short-term or continuous exposures (Table 1). In the controls, 19.2% of the seeded monocytes adhered after 2 h of culturing and with doxorubicin, adherence ranged from 17.4% to 20.5%. Thus, doxorubicin did not affect cell recovery in adherent cultures. The total amount of cells harvested after 10 days was over 100% at all concentrations of drug studied. Cell recovery and viability of the non-adherent monocytes were 83.6 and over 95%, respectively (Table 1).

Effects of doxorubicin on enzyme activities

After culture without doxorubicin for 10 days in plastic tubes, G-6-PDH activity increased 25 times compared with the level observed after 2 h culture. The corresponding figures for PHI and ICDH were 6 and 4 times, respectively. ACP and NAG activity increased 33 and 10 times. Compared with 1 h non-adherent incubation (polypropylene tubes, 37°C), enzyme activities of monocytes cultured in Teflon foil bags after 10 days were increased as follows: G-6-PDH, 27 times; PHI, 5 times; ICDH, 8 times; ACP, 19 times; and NAG, 28 times (Table 2).

In the presence of doxorubicin, in short-term (750 and 1500 ng/ml) or continuous exposure (75 ng/ml), enzyme activities of adherent or non-adherent monocytes were similar to the control values (Table 2).

Effect of doxorubicin on FcR, CD14 and HLA-DR expression

We characterized the culture macrophages, which were continuously exposed to doxorubicin 75 ng/ml or underwent short-term exposure to 750 or 1500 ng/ml, before culturing. All schedules and doses of doxorubicin diminished the expression of FcRI (32–45%), FcRII (10–26%), CD14 (20–37%), and HLA-DR (25–34%) (Fig. 1). The reductions were all significant (t test) at $P < 0.05$, with the exception of the effect on the FcRII expression after continuous exposure ($P < 0.154$). Expression of FcRIII was not affected, because this receptor was not present on our 10 day cultured macrophages.

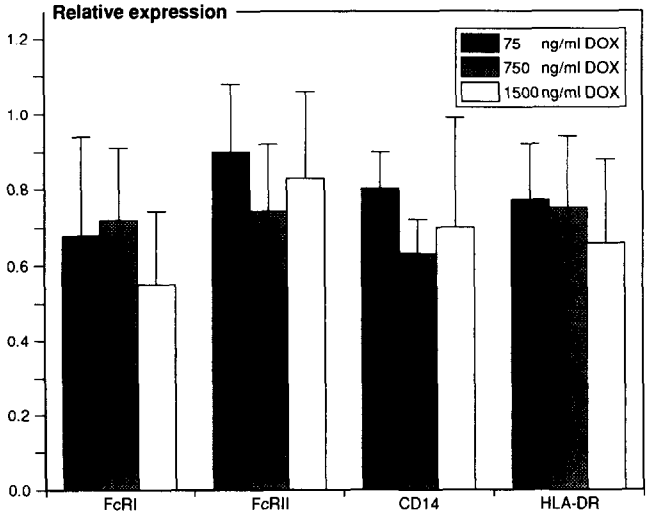

Fig. 1. Expression of FcRI, FcRII, CD14, and HLA-DR on monocyte-derived macrophages. Monocytes were cultured for 10 days in Teflon foil bags without drug or with continuous exposure to doxorubicin 75 ng/ml, or after short-term exposures to 750 or 1500 ng/ml. Relative expression = doxorubicin-treated:non-treated ratio (n = 5, error bar = S.D.). DOX = doxorubicin.

DISCUSSION

As part of the investigation of the influence of cytostatic agents on the immune system, we evaluated the effects of doxorubicin on human monocytes in adherent and non-adherent cultures. We measured extracellular and intracellular drug concentrations, because doxorubicin can be rapidly metabolized into doxorubicinol and doxorubicinone [23]. Since doxorubicinone is not cytotoxic [24] and the cytotoxic potential of doxorubicinol is only a tenth that of the parent compound [25], we needed to know whether formation of these derivatives could be excluded. In our systems, no metabolites were found. In many *in vitro* experiments cell death is related to cellular doxorubicin concentrations [26]. It is not clear whether free radical formation, intercalation of doxorubicin into DNA, induction of DNA breaks and chromosomal aberrations, or drug-induced alterations of cell membranes actually kill the cell.

At high concentrations of doxorubicin obtainable only *in vitro*, negative effects were seen on cell recovery, viability, and morphology after 10 days of culture. To mimic the clinical situation, we used two schedules of non-toxic incubation: continuous exposure to doxorubicin 75 ng/ml (akin to continuous infusion), and short-term exposure to 750 or 1500 ng/ml before culture (akin to bolus infusion).

Doxorubicin did not influence the adherent capacity or yield of the cultured monocytes. Our observation that adherence was unaffected was different from the results described by Athlin and Domellof [27]. They described a significant reduction of monocyte glass attachment 1 week after patients had been treated with combination chemotherapy of fluorouracil, doxorubicin, and mitomycin. However, these discrepancies can be explained by the different drugs administered, and *in vivo* versus *in vitro* conditions. Another point is that, 1 week after treatment, Athlin and Domellof were looking at a monocyte population that was not present in the peripheral blood at the time of the combination chemotherapy. When these patients were treated, this cell population were still precursor monocytes. Patients with solid tumors also showed a marked decrease in yield of adherent macrophages after a week of culture [28, 29]. In our study, the amount of adhered cells increased slightly with the time of culture independently of the doxorubicin treatment. After 10 days the overall yield of suspension-cultured macrophages was more than 2.5 times higher than plastic-cultured macrophages. These observations indicate the existence of adherent and non-adherent monocytes, both capable of maturation to macrophages, and suggest that at least some monocytes can become adherent macrophages as a consequence of maturation and age. Our finding that after 2 h nearly a fifth of the plastic-seeded monocytes adhered contradicts our earlier report [18]. Previously, we found about half the cells adhered after 2 h, however, the yield of adherent macrophages after 15 days was 70% (20) of those originally seeded, which is similar to our yield after 10 days [31.8% (12.1)]. It is not easy to explain these differences because we used the same conditions on both occasions. Depending on the conditions there is considerable variation in the adherent yield, resulting in marked differences in the relative amounts of adherent and non-adherent cells. Results of studies of monocyte function should be critically regarded in this respect. Monocyte isolation by counterflow centrifugation [17] has an advantage here since selection is minimal.

There are reports of changes in enzymes and receptors as indicators of the *in vitro* maturation of non-treated monocytes [18, 21, 30–33]. No information is, however, available about the effects of doxorubicin on maturation as reflected in the enzyme levels of highly purified and *in vitro* cultured monocytes. We found that in the presence of doxorubicin, the intracellular level of G-6-PDH was unaltered, which indicates that hexose monophosphate shunt activity was similar to unexposed cells. The activities of PHI, of the glycolytic pathway, and of ICDH, of the Krebs' cycle, were also unaffected. NAG and ACP activity, important for the hydrolytic degradation of glycoproteins, mucopolysaccharides, and glycolipids, remained the same when cells were exposed to doxorubicin continuously or in a short-term schedule.

Tritton and Yee [34] showed that doxorubicin can act solely by interaction with the cell surface, so it may be important to consider the cell surface as a target. We examined possible alterations of the cell membrane by doxorubicin by study of the expression of FcRI, FcRII, FcRIII, CD14, and HLA-DR. FcRs and HLA antigens can be affected by biological response modifiers [35, 36]. Modulation of CD14, a membrane protein expressed on the surface of mature monocytes and macrophages [14, 15], has not been previously described. We found that doxorubicin caused a slight but significant decrease in FcRI, FcRII, CD14, and HLA-DR expression on *in vitro* cultured monocytes (with the exception of FcRII in the continuous exposure experiments). That FcRIII expression was not measurable in our study can be explained by the observation of Clarkson and Ory [12] that FcRIII, which is not present on monocytes, will appear on macrophages in measurable quantities after 14 days of culture. How doxorubicin modulates the expression of surface molecules and whether this will affect function are not known. Our preliminary experiments indicate that doxorubicin does not influence FcRI and FcRII mediated, antibody-dependent cellular cytotoxicity. Alterations in membrane molecules will be counteracted by *de novo* synthesis of the original membrane molecules, unless inhibited by DNA intercalation.

1. Somers SD, Johnson WJ, Adams DO. Destruction of tumor cells by macrophages: mechanisms of recognition and lysis and their regulation. In: Herberman RB, ed. *Cancer Immunology: Innovative Approaches to Therapy*. Boston, Martinus Nijhoff, 1986, 69–122.
2. Evans R. Macrophages and neoplasms: new insights and their implications in tumour biology. *Cancer Metastasis Rev* 1984, **1**, 227–239.
3. Ogawa M, Muggia FM, Rozencweig M. *Adriamycin, Its Expanding Role in Cancer Treatment*. International Congress Series No. 629. Amsterdam, Excerpta Medica, 1984.
4. Speth PAJ, Van Hoesel QGCM, Haanen C. Clinical pharmacokinetics of doxorubicin. *Clin Pharmacokinet* 1988, **15**, 15–31.
5. Oldham R. Biotherapy: the 4th modality of cancer treatment. *J Cell Physiol* (Suppl.) 1986, **4**, 91–99.
6. Eppstein DA, Van der Pas RA, Marsh YV, Bruno NA, Kurahara CG. Enhanced antitumor efficacy of Adriamycin in combination with interferon-γ: effects on macrophages. *J Interferon Res* 1988, **8**, 263–273.
7. Creagan ET, Long HJ, Frytak S, Moertel CG. Recombinant leukocyte A interferon with doxorubicin: a phase I study in advanced solid neoplasms and implications for hepatocellular carcinoma. *Cancer* 1988, **61**, 19–22.
8. Mantovani A. The interaction of cancer chemotherapy agents with mononuclear phagocytes. *Adv Pharmacol Chemother* 1982, **19**, 35–66.
9. Fanger MW, Shen L, Graziano RF, Guyre PM. Cytotoxicity mediated by human Fc receptors for IgG. *Immunol Today* 1989, **10**, 92–99.
10. Kinet J-P. Antibody–cell interactions: Fc receptors. *Cell* 1989, **57**, 351–354.
11. Adams DO, Hall T, Steplewski Z, Korpowski H. Tumors undergoing rejection induced by monoclonal antibodies of the Ig2a isotype contain increased numbers of macrophages activated for a distinctive form of antibody-dependent cytolysis. *Proc Natl Acad Sci USA* 1984, **81**, 3506–3510.
12. Clarkson S, Ory PA. CD16. Developmentally regulated IgG Fc receptors on cultured human monocytes. *J Exp Med* 1988, **167**, 408–417.
13. Van de Winkel JGJ, Boonen GJJC, Janssen PLW, Vlug A, Hogg N, Tax WJM. Activity of two types of Fc receptors, FcγRI and FcγRII, in human monocyte cytotoxicity to sensitized erythrocytes. *Scand J Immunol* 1989, **29**, 23–31.
14. Simmons DL, Tan S, Tenen DG, Nicholson-Weller A, Seed B. Monocyte antigen CD14 is a phospholipid anchored membrane protein. *Blood* 1989, **73**, 284–289.
15. Bogman MJJT, Dooper PhMM, Assmann KHM *et al.* Immunohistologic staining of renal transplantant biopsies with monoclonal antibody WT14: a new parameter for the diagnosis of rejection. *Transplant Proc* 1989, **21**, 1886–1887.
16. Unanue ER, Allen PM. The basis for the immunoregulatory role of macrophages and other accessory cells. *Science* 1987, **236**, 551–553.
17. De Mulder PHM, Wessels JMC, Rosenbrand DA, Smeulders JBJM, Wagener DJTh, Haanen C. Monocyte purification with counterflow centrifugation monitored by continuous flow cytometry. *J Immunol Methods* 1981, **47**, 31–38.
18. De Mulder PHM, Van Rennes H, Mier PD, Bergers ME, De Pauw BE, Haanen C. Characterization of monocyte maturation in adherent and non-adherent cultures and its application to study monocyte differentiation in Hodgkin's disease. *Clin Exp Immunol* 1983, **54**, 681–688.
19. Speth PAJ, Linssen PCM, Boezeman JBM, Wessels JMC, Haanen C. Rapid quantitative determination of four anthracyclines and their main metabolites in human nucleated hematopoietic cells. *J Chromatogr* 1986, **377**, 415–422.
20. Moore M, Rossman MD, Schreiber AD, Douglas SD. DR framework antigens and 6303 antigens on human peripheral blood monocytes and alveolar macrophages. *Clin Immunol Immunopathol* 1983, **29**, 119–128.
21. De Mulder PHM, Mier PD, De Pauw BE *et al.* Altered intracellular enzyme activity of monocytes and lymphocytes in Hodgkin's disease. *Eur J Cancer Clin Oncol* 1983, **19**, 333–337.
22. Mier PD, Van Rennes H, Van Erp PEJ, Roelfzema H. Cutaneous silaidase. *J Invest Dermatol* 1982, **78**, 267–269.
23. Takanashi S, Bachur NR. Adriamycin metabolism in man, evidence for urinary metabolites. *Drug Metab Dispos* 1976, **4**, 79–87.
24. Bachur NR, Steele M, Meriwether WD, Hildebrand RC. Cellular pharmacodynamics of several anthracycline antibiotics. *J Med Chem* 1976, **19**, 651–654.
25. Dessypyris EN, Brenner DE, Krantz SBG, Hande KR. Toxicity of Adriamycin metabolites to human bone marrow erythroid and myeloid progenitors *in vitro*. *Proc Am Assoc Cancer Res* 1984, **25**, abstr. 1203.
26. Speth PAJ, Raijmakers RAP, Boezeman JBM *et al. In vivo* cellular Adriamycin concentrations related to growth inhibition of normal and leukemic bone marrow cells. *Eur J Cancer Clin Oncol* 1988, **24**, 667–674.
27. Athlin L, Domellof L. Response of human monocyte phagocytosis to FAM (fluorouracil, Adriamycin, mitomycin). *Acta Chir Scand* 1987, **153**, 279–281.
28. Taylor SA, Currie GA. Monocyte maturation and prognosis in primary breast cancer. *Br Med J* 1979, **6170**, 1050–1051.
29. Krishman EC, Henon CD, Krishman L, Jewell WR. Deficiency in maturation process of macrophages in human cancer. *J Natl Cancer Inst* 1980, **65**, 273–276.
30. Zuckerman SH, Ackerman SK, Douglas SD. Longterm human peripheral blood monocyte cultures: establishment, metabolism and morphology of primary human monocyte–macrophage cell cultures. *Immunology* 1979, **38**, 401–411.
31. Musson RA Shafran H, Henson PM. Intracellular levels and stimulated release of lysosomal enzymes from human peripheral blood monocytes and monocyte-derived macrophages. *J Retic Endothel Soc* 1980, **28**, 249–263.
32. Johnson WD, Mei D, Cohn ZA. The separation, long-term cultivation, and maturation of the human monocyte. *J Exp Med* 1977, **146**, 1613–1626.

33. Hammerström J. Human macrophage differentiation *in vivo* and *in vitro*. A comparison of human peritoneal macrophages and monocytes. *Acta Path Microbiol Scan Sect C* 1979, **87**, 120–133.
34. Tritton TR, Yee G. The anticancer agent Adriamycin can be actively cytotoxic without entering the cells. *Science* 1982, **217**, 248–250.
35. Guyre PM, Morganelli PM, Miller R. Recombinant immune interferon increases immunoglobulin G Fc receptors on cultured human mononuclear phagocytes. *J Clin Invest* 1983, **72**, 393–397.
36. Liesveld JL, Abboud CN, Looney RJ, Ryan DH, Brennan JK. Expression of IgG Fc receptors in myeloid leukemic cell lines. Effect of colony- stimulating factors and cytokines. *J Immunol* 1988, **140**, 1527–1533.

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Potentiation by Phenylbisbenzimidazoles of Cytotoxicity of Anticancer Drugs Directed against Topoisomerase II

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Analogues of the phenylbisbenzimidazole dye pibenzimol bind tightly to the minor groove of DNA. A clonogenic assay has been used to investigate the effects of these compounds on the cytotoxicity of the topoisomerase II directed anti-cancer drugs amsacrine, CI-921 (an amsacrine analogue), acridine carboxamide, etoposide and doxorubicin. Although pibenzimol itself was inactive, several of its analogues reduced the toxicity of etoposide, amsacrine and CI-921 towards a Lewis lung mouse tumour line at concentrations between 1 and 20 μmol/l. Doxorubicin cytotoxicity was unaffected, suggesting that this drug has a distinct mechanism of action. At concentrations below 1 μmol/l, some of these dyes potentiated the cytotoxicity of etoposide and CI-921 towards Lewis lung cells. Potentiation of CI-921 activity was also found with the human tumour lines HT29 (colon), SW620 (colon) and FME (melanoma). Novel treatments may arise from the potentiation of topoisomerase II directed cytotoxicity.
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INTRODUCTION

TOPOISOMERASE II (topo II) is essential for DNA replication and is believed to be the target of anti-cancer drugs such as the epipodophyllotoxins, the anthracyclines and synthetic compounds, including amsacrine, its analogue CI-921, N-[2-(dimethylamino)ethyl]acridine-4-carboxamide (acridine carboxamide) and mitoxantrone [1, 2]. Previously we found that acridine carboxamide had the unusual property of inhibiting its own cytotoxic action at high concentration [3, 4]. We then showed that 9-aminoacridine and certain related DNA intercalators can chemoprotect against the cytotoxic effects of etoposide, amsacrine, CI-921 and acridine carboxamide both *in vitro* and *in vivo* [5].

We have used a series of Hoechst dyes with a clonogenic assay to investigate the modulation of cytotoxicity in cultured murine Lewis lung (LLTC) carcinoma cells. Phenylbisbenzimidazole dyes bind to DNA [6]. X-Ray crystallography reveals that pibenzimol (H33258) binds to the minor groove of double-stranded B-DNA and is selective for adenine–thymine rich regions [7]. This compound affects the chromosomes of growing cells, inhibiting the condensation of adenine–thymine rich regions [8] and it also inhibits the action of both topo I [9] and topo II [10] in cell-free systems. The effect of pibenzimol on murine leukaemia has prompted a phase I trial [11]. However, along with other minor groove DNA binding compounds [12], pibenzimol has not yet found a place in current cancer chemotherapy.

In this study we have investigated whether DNA minor groove binders can modulate the activity of topo II directed agents.

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