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Melanin synthesis and the action of L-dopa and 3,4-dihydroxybenzylamine in human melanoma cells

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Summary. The toxicity and selectivity of 3,4-dihydroxybenzylamine (DHBA), an experimental antimelanoma agent that cannot enter the melanin pathway, broadly paralleled that of L-dopa in a panel of human melanoma cell lines sensitive or resistant to the latter drug. A human retinoblastoma cell line was found to be sensitive to both compounds. The toxicity and selectivity of both catechols were associated with inhibition of DNA synthesis; DHBA was more potent yet allowed a much greater degree of recovery compared with an equitoxic level of dopa. Dopa and DHBA had similar, dose-dependent effects on the cell cycle, arresting cells in S phase at low doses and in G_1 at high doses. Replication of the DNA virus adenovirus was found to be inhibited by both agents. There was no difference between sensitive and resistant cell lines in the manganese or copper/zinc forms of superoxide dismutase, or in iron content and iron-binding capacity. Catechol toxicity was inhibited by the hydrogen peroxide scavenging agents pyruvate and methaemoglobin. Sensitivity to catechols did not correlate with melanin or tyrosinase content, rate of incorporation of tyrosine or dopa, intracellular levels of phenylalanine or tyrosine, or binding of a new monoclonal antibody directed against a melanosomal protein. These results indicate that DHBA and dopa exhibit selective toxicity for neural crest tumor cells independently of the melanisation pathway and of the superoxide scavenging system.

Introduction

L-Dopa and other catechols have shown activity in vivo against rodent tumors [30, 31, 36] including melanoma [29], and selective toxicity to melanoma cells in vitro [19, 29]. The variety of effects of catechols on cells has made it difficult to distinguish between mechanisms of toxicity based on quinones or active oxygen species as the reactive intermediates [6, 8, 12, 18, 22, 35]. Murine lymphoid cells were arrested by dopa methyl ester in G_1/S with inhibition of DNA synthesis [32], due possibly to inhibition of DNA polymerase [2] or ribonucleotide reductase [1]. DNA breaks and inactivation of adenovirus by dopa have also been reported [18, 19, 38]. In a study of factors which affect dopa toxicity in culture medium containing 10% fetal calf serum [18], exogenous superoxide dismutase and catalase were found to decrease toxicity whereas inhibitors of these enzymes had the converse effect. The D isomers of dopa and penicillamine, which are not taken up by cells, had the same toxicity as the L isomers. The early events of dopa toxicity were therefore considered to include generation of oxygen radicals outside the cell, either from autooxidation of dopa to melanin or from melanin itself. The inhibition of dopa toxicity observed at physiological oxygen levels and at high cell density indicated that dopa would be much less potent in vivo than in vitro.

DHBA has shown activity against rodent tumours in vivo [32], and its action resembles in some respects that of known inhibitors of DNA synthesis [3]. On the basis of being a more effective inhibitor of DNA synthesis in a cell-free system, DHBA has been proposed [2, 32, 33, 35] as an alternative agent to dopa for melanoma therapy. DHBA, which is a substrate for tyrosinase but cannot cyclise and polymerise as dopa does, required the presence of tyrosinase for inhibition of DNA polymerase [34] and for toxicity in B16 mouse melanoma cells [24]. Recently, it was suggested that the toxicity of hydroquinone [20] and cysteinylphenols [37] in melanoma cells occurred via tyrosinase-mediated oxidation. We previously found that dopa sensitivity was not related to melanin or tyrosinase levels in human cells [18]. We have therefore directly compared the action of dopa and DHBA in a panel of human tumor cell lines where the melanin biosynthetic pathway and sensitivity to redox-inactive inhibitors of DNA synthesis have been characterised in more detail; this study includes a new melanoma line with much higher melanogenic activity than those available previously.

Materials and methods

The origins of HeLa-S3 and the human melanoma cell lines MM96E, MM96L, MM127 and MM253c1 have been described elsewhere [18, 19]. The human retinoblastoma line RT-1 [27] was obtained from Dr. P. Smith of this Institute. Cells were cultured at 37° C in vessels flushed with 90% nitrogen/5% oxygen/5% CO_2 in Roswell Park Memorial Institute Medium 1640 (Commonwealth Serum Laboratories, Australia) containing streptomycin (100 μg/ml), penicillin (100 i.u./ml), 4-(2-hydroxyethyl)-1-piperazine-

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ethanesulphonic acid (3 m*M*) and 10% (v/v) fetal calf serum. Routine assays of cell lines and the adenovirus pool for *Mycoplasma* by agar culture were negative. Cell survival was determined as previously described [19] by treating duplicate cultures (3×10^3 per 16-mm well) seeded 24 h previously. After continuous treatment for 6 days, cells were labelled with 2 μCi/ml [^{3}H-methyl]-thymidine (40 Ci/mmol; Radiochemical Centre, Amersham, UK) for 2–4 h, detached with 0.02% trypsin and washed with water onto glass fibre discs for liquid scintillation counting. Curves were plotted for survival (calculated as % control cpm), determined at five doses for each cell line. Survivals were compared on the basis of the D_{37}, the dose at which survival was 37%. Stock solutions of drugs were freshly prepared in culture medium for each experiment. The t½ for autoxidation of dopa and DHBA in culture medium was determined from semilogarithmic plots of the increase in absorbance at 320 nm.

Incorporation of isotopically labelled dopa (10 μ*M*) and tyrosinase (0.2 μ*M*) was determined by adding the isotopes (Radiochemical Centre, Amersham, UK) to duplicate cultures (5×10^4 cells/16 mm well) in culture medium and harvesting at various times as described above. The isotopes used were L-[3-^{14}C]-dopa (5 Ci/mol), L-[1-^{14}C]-dopa (5 Ci/mol), L-[U-^{14}C]-tyrosinase (522 Ci/mol) and L-[3,5-^{3}H]-tyrosine (49 Ci/mmol). The filters were treated with 0.1 ml Soluene 350 (Packard Instruments, Zurich, Switzerland) prior to liquid scintillation counting of dpm. Intracellular pools of phenylalanine and tyrosine were determined by HPLC after derivatisation of protein-free cell lysates with phenylisothiocyanate [7]. The lysates were prepared by suspension of 10^7 cells in 1 ml H_2O and immediate centrifugation in an Amicon Centricon 10 microconcentrator (Danvers, Mass). Cell volumes were determined with a particle counter (Particle Data, Elmhurst, Ill). Tyrosinase activities of cells lysed with 1% Triton X-100 were measured in 7.6 m*M* dopa-50 m*M* phosphate buffer, pH 6.8 by following the increase in absorbance at 490 nm [4]. Melanin was determined by dissolving a pellet of 10^6 cells in 1 ml Soluene 350 (Packard Instruments) and reading the absorbance at 400 nm. Dopa-melanin (Sigma, St. Louis, Mo) was used as the standard.

Reactivity of methanol-fixed cells (10^4/6 mm well) with the monoclonal antibody MoAb 1C11 was determined by sequential incubations with MoAb 1C11 hybridoma culture medium, alkaline phosphatase-linked goat antimouse antibody (Silenus Laboratories, Melbourne, Australia) diluted 1/1000 in phosphate-buffered saline, and 5 m*M* *p*-nitrophenyl phosphate in 0.1 *M* diethanolamine-5 m*M* $MgCl_2$, pH 10.5. The change in absorbance at 405 nm was read with an ELISA plate reader (Model EL310, Bio-Tek Instruments, Burlington, Va). MoAb 1C11 was derived using tyrosinase purified from MM96E as the immunogen and was shown by immunogold staining to react with a melanosomal antigen (M. Mc Ewan et.al. submitted for publication).

Superoxide dismutase activity was determined directly from the decrease in A_{250} of potassium superoxide at pH 9.5, as described by Marklund [14]. The rate was determined from linear first-order plots of A_{250} vs time and corrected for spontaneous decay. One unit of activity was defined as a rate of 0.1 s^{-1} under the above conditions [14]. Iron and iron-binding capacity were determined with ferrozine [21] using a Sigma diagnostic kit no. 565.

Nucleic acid synthesis was determined by treating duplicate monolayers (5×10^4 cells/16-mm well) with dopa or DHBA for 1 h, washed twice with medium and at various times pulshed for 1 h with a mixture of 1 μCi/ml [5-^{3}H]-uridine (40 Ci/mmol) and 0.05 μCi/ml [methyl-^{14}C]-thymidine (20 Ci/mol). Cells were harvested and processed as described above for liquid scintillation counting of dpm.

Ten-fold dilutions of adenovirus 5 [18] were used to infect duplicate cultures (5×10^3 cells/microtitre well) seeded 24 h previously. After 1 h, the drug was added and allowed to remain on the cells for 48 h. The plates were then washed with phosphate-buffered saline, fixed with methanol and stained for detection of viral antigen as previously described [18], using successive treatments with adenovirus antibody, peroxidase-labelled protein A and peroxidase substrate. One infecting dose (ID) was defined as the amount of virus required to produce one stained cell.

The effect of drugs in cell cycle progression was determined by DNA flow cytometry [28]. Cultures seeded 24 h previously (10^5 cells/60-mm plate) were treated for 24 h, detached with 0.2 mg/ml trypsin in PBS and fixed in 25% ethanol at 0°. After staining with a mixture of propidium iodide (50 mg/ml), Triton X-100 (0.2%) and RNase A (1 mg/ml) in PBS, the cells were analysed with a Becton Dickinson FACS IV flow cytometer operated at 488 nm. The proportion of cells in each phase of the cell cycle was calculated from the relevant areas (G_1, S and G_2) of the DNA histogram.

Results

Cell survival

The properties of the redox-sensitive melanoma line MM96L were compared with those of HeLa, a resistant line having a similar cell volume (1500–1700 μm^3) and doubling time (24 h). As reported previously [19], MM96L was very sensitive to killing by dopa compared with HeLa ($P < 0.01$ for D_{37} in Table 1), as was evident mainly by the lack of a shoulder on the survival curve (Fig. 1 A). The D_{37} values were higher than found previously, presumably because the present studies were carried out in 5% oxygen in order to simulate physiological conditions more closely. The survival curve for the retinoblastoma cell line RT-1 was similar to that of MM96L (Fig. 1 A). DHBA paralleled dopa in its relative toxicity to MM96L and HeLa

Table 1. Toxicity of dopa and DHBA in human tumor cell lines

Cell line	Sensitivity to hydroxyurea and methotrexate[a]	D_{37} (μ*M*)	
		Dopa	DHBA
HeLa	+	80 ± 12[c]	213 ± 21
CI-80-13S	NT[b]	108	91
RT-1	NT	17 ± 3	20 ± 2
MM96L	–	23 ± 2	27 ± 5
MM127	–	29	17
MM229	–	5.2	NT
MM253c1	+	76 ± 17	55 ± 11
MM418	–	71 ± 9	47 ± 0.3

[a] Reference [15]
[b] Not tested
[c] Mean ± SD (n = 3-5)

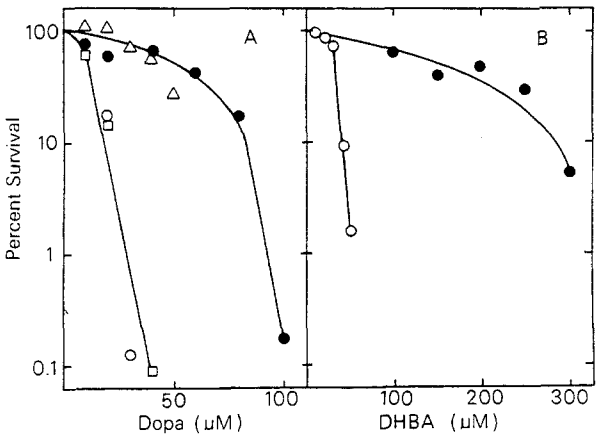

Fig. 1 A, B. Dose responses of cell survival. **A** Dopa ○, MM96L; □, RT-1; ●, HeLa; △, MM96L treated simultaneously with 1 m*M* pyruvate; **B** DHBA: ○, MM96L; ●, HeLa. *Points* are means of duplicates

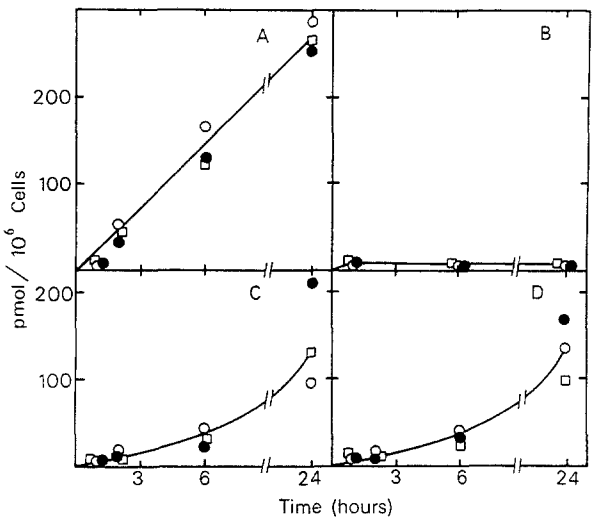

Fig. 2 A–D. Incorporation of isotopically-labelled dopa (10 μ*M*) and tyrosine (0.2 μ*M*) by tumour cells. ○, MM96L; □, MM96E; ●, HeLa. **A** [3-^{14}C]-Dopa; **B** [1-^{14}C]-dopa; **C** [U-^{14}C]-tyrosine; **D** [3,5-^{3}H]-tyrosine. *Points* are means of duplicates

($P < 0.001$), except that a higher level was required to achieve the same level of killing in HeLa cells (Fig. 1 B). DHBA was slightly more stable in culture medium than dopa (½ 26 h and 19 h, respectively). The toxicity of dopa was greatly reduced in the presence of 1 m*M* pyruvate (Fig. 1 A) or methaemoglobin (100 μg/ml). These compounds also inhibited the toxicity of DHBA (D_{37} for MM96L 45 μ*M* respectively). A dose-response study of methaemoglobin using MM96L cells treated with 45 μ*M* dopa indicated that a two- to five-fold inhibition of toxicity occurred at levels found in some batches of fetal calf serum (50–100 μg/ml).

Eight human tumour cell lines compared on the basis of D_{37} (Table 1) fell into two distinct groups, dopa-sensitive ($D_{37} < 30$ μ*M*) and dopa-resistant ($D_{37} > 70$ μ*M*). The four lines resistant to dopa were also resistant to DHBA, although the D_{37} values for DHBA were spread over a wider range than those for dopa. No correlation was found with resistance to hydroxyurea or methotrexate. It was of interest that DHBA was less toxic to HeLa than dopa but more toxic in the MM253c1 line. There was no correlation between catechol sensitivity and melanogenic activity as judged by tyrosinase or melanin contents (Table 2).

Properties of redox-sensitive and redox-resistant cell lines

Previous studies [18, 19] ruled out a quantitative relationship between catechol sensitivity and the activity of the melanisation pathway, but did not address the possibility of a qualitative difference between even low tyrosinase activity (as in MM96L, for example) and no activity (as in HeLa). The rates of incorporation (as distinct from uptake) of melanin precursors were therefore compared. The results plotted in Fig. 2 A showed that side-chain-labelled dopa was incorporated at the same rate in MM96L, in a more melanised subline (MM96E), and in HeLa (correlation coefficient for the combined data was 0.96). The carboxyl label of dopa (Fig. 2 B) was not incorporated, presumably because of rapid metabolism. Side-chain- or ring-labelled tyrosine was incorporated to an equal extent in each cell line (Fig. 2 C, D; correlation coefficients 0.90 and 0.96). It should be noted that although 0.2 μ*M* labelled tyrosine was added for these experiments, the total concentration was that of RPMI culture medium (140 μ*M*) plus that released by proteolysis of serum; thus, the absolute amount of tyrosine incorporation cannot be compared with that of dopa. Direct measurement of tyrosine and phenylalanine pools revealed wide variation between cell lines, HeLa and MM96L having much lower concentrations than less rapidly proliferating cell lines (Table 2). The phenylalanine/tyrosine ratio, reported previously in

Table 2. Markers of melanogenesis in human cells

Cell	Tyrosinase (pmol/min per 10^6 cells)	Melanin (pg/ml)	Phenyl-alanine (m*M*)	Tyrosine (m*M*)	Phe/Tyr	MoAb 1C11 (Abs/40 min)		
						Control	Dopa[a]	DHBA[a]
HeLa	<0.5	<0.05	0.59	0.88	0.66	0.035±0.008[b]	0.025±0.005	0.030±0.003
CI-80-13S	<0.5	<0.05	2.4	3.7	0.65			
Melanoma								
MM96L	126	0.2	1.3	1.8	0.71	0.504±0.134	0.299±0.015	0.269±0.093
MM253c1	3	<0.05	11.8	17.7	0.66	0.151±0.130	0.145±0.030	0.119±0.070
MM418	200	13	6.5	4.2	1.5	0.450±0.112	0.412±0.095	0.424±0.068
MM229	<0.5	<0.05	8.8	13.5	0.065	0.026±0.002	0.028±0.005	0.027±0.002
MM127	<0.5	<0.05	12.8	19.6	0.65			

[a] 0.05 m*M* for 16 h

[b] Mean and SD ($n = 2$)

HeLa as 0.65 [23], was relatively constant (0.5–0.7) in all lines except for the highly melanised MM418 line, where the ratio was 1.5. The phenylalanine level in MM418 (1.9 m*M*) was close to the mean for other lines (1.8 m*M*, excluding MM96L and HeLa), whereas the tyrosine level (1.2 m*M*) was much lower than the mean (2.7 m*M*).

The melanosome-directed MoAb 1C11 reacted strongly with the amelanotic MM253cl cell line as well as with the lightly melanised MM96L line (Table 2). No correlation of MoAb 1C11 reactivity was found with sensitivity to catechols, nor was antigenicity affected by drug treatment except for a moderate inhibition in MM96L cells.

Cellular superoxide dismutase was measured directly from first-order plots of the rate of decomposition of superoxide ion. The activity of the manganese form of the enzyme was distinguished from that of the copper/zinc form by inhibition of the latter wih potassium cyanide [29]. The results (Table 3) showed no difference between MM96L and HeLa in the activity of either enzyme ($P > 0.05$). Treatment with 5 m*M* dopa or DHBA for 24 h had no significant effect on enzyme activity (92% and 97% of control cells, respectively). The catalase activity in these cell lines had previously been shown to be similar [18]. Since catechols may inactive catalase [9], enzyme activity was determined in cells treated with 5 m*M* dopa for 24 h. No decrease in activity was found (90% of the activity in control cells).

The possibility of iron complexes such as transferrin participating in redox reactions [11] prompted a comparison of cellular iron content and iron-binding capacity. No difference was found between MM96L and HeLa (Table 3; $P > 0.05$).

Effect of dopa and DHBA on DNA and RNA synthesis

Because catechol toxicity decreased with increasing cell density [19], preliminary experiments were carried out to establish suitable conditions for the determination of nucleic acid synthesis. At the cell density used for these experiments, a 1-h treatment with 20 m*M* dopa or DHBA led to 10%–30% survival in MM96L and 90%–100% in HeLa. As shown in Fig. 3 A, treatment with 2 m*M* or 20 m*M* dopa inhibited DNA synthesis more in MM96L than in HeLa cells ($P < 0.05$ for the 0- to 6-h points). No significant recovery was observed during the subsequent 24 h. DHBA, on the other hand, showed a stronger initial inhibition of DNA synthesis compared with dopa but a considerable degree of recovery, that with 2 m*M* DHBA treatment exceeding the control value in both cell lines after 6 h (Fig. 3 B). Similar results were obtained in repeated experiments. The magnitude of the 2 m*M* DHBA enhancement varied somewhat but was always >100%, being 139%±14% for MM96L and 135% ±9% for HeLa (mean and SE for four

Table 3. Superoxide dismutase activity, iron content and iron-binding capacity of MM96L and HeLa cells

Cell line	Superoxide dismutase (units/10^6 cells)			Iron (ng/10^6 cells)	
	Total	Mn[a]	Cu/Zn[b]	Total	Iron-binding capacity
MM96L	20.4±4.1[c]	11.9±4.2	8.5	37	346±31
HeLa	17.2±3.6	11.4±0.6	5.8	37	321±57

[a] Determined in the presence of 8 m*M* KCN
[b] Total minus Mn activity
[c] Mean and SD for 5 determinations

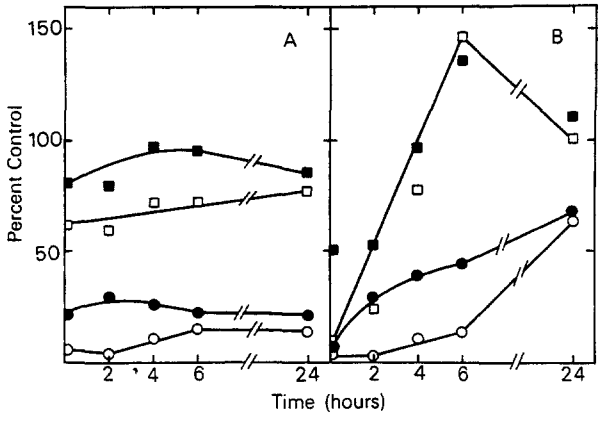

Fig. 3 A, B. Temporal effect of 1-h treatment with dopa or DHBA on DNA synthesis as judged by pulse labelling with ^{14}C-thymidine. □, MM96L and 2 m*M* ■, HeLa and 2 m*M* ○, MM96L and 20 m*M* ●, HeLa and 20 m*M*. **A** Dopa; **B** DHBA. *Points* are means of duplicates

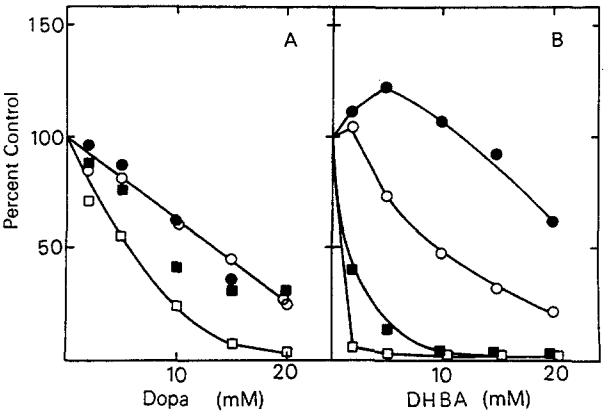

Fig. 4. A Dopa and **B** DHBA dose-responses of DNA synthesis after a 1-h treatment. □, MM96L at 0 h; ■, HeLa at 0 h: ○, MM96L at 6 h; ●, HeLa at 6 h. *Points* are means of duplicates

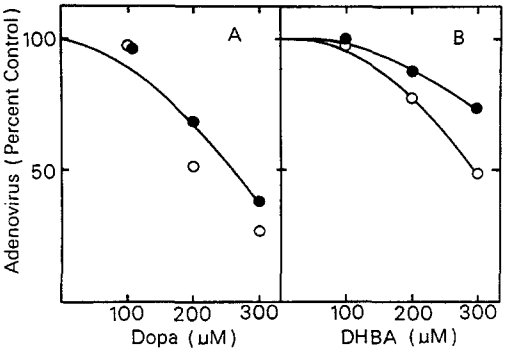

Fig. 5 A, B. Replication of adenovirus in cells during treatment with dopa **(A)** or DHBA **(B)**. ○, MM96L; ●, HeLa. *Points* are means of duplicates

Table 4. Effect of dopa and DHBA on cell cycle progression

Cell line	Cell phase	Percentage of cells in each phase after 24 h treatment				
		Control	Dopa		DHBA	
			1 m*M*	5 m*M*	1 m*M*	5 m*M*
HeLa	G_1	64	54	80	49	78
	S	17	23	8.7	34	9.4
	G_2	19	23	12	17	13
MM96L	G_1	62	69	84	52	74
	S	21	22	13	37	18
	G_2	17	9.2	3.7	11	8.9

experiments). MM96L was inhibited more than HeLa by 20 m*M* DHBA up to 4 h ($P < 0.05$). The dopa dose-response curve for DNA inhibition at two different times (Fig. 4A) showed that a consistent difference between MM96L and HeLa was only found immediately after treatment ($P < 0.05$ for 10–20 m*M* dopa). The dose-response curve from DHBA (Fig. 4B) confirmed the ability of low levels of DHBA to immediately inhibit DNA synthesis and showed that the apparent stimulation of DNA synthesis 6 h after treatment was maximal in the nontoxic dose range of 2–5 m*M*. As expected from the time-response study, MM96L was affected more than HeLa 6 h after treatment with 10–20 m*M* DHBA ($P < 0.05$). Measurement of RNA synthesis by incorporation of ^{3}H-uridine was less reproducible than for DNA synthesis. In repeated experiments, no significant inhibition by dopa or DHBA was detected using the above conditions.

Replication of adenovirus was used as an independent test for inhibition of DNA synthesis in drug-treated cells. The dose and time relationships differed from those in the thymidine incorporation experiment because of the longer time needed for replication of viral DNA. The results in Fig. 5 indicate partial inhibition of viral replication in MM96L and HeLa by continuous treatment with dopa and DHBA; the slight selectively against MM96L compared with HeLa using DHBA (Fig. 5B) did not reach statistical significance in repeated experiments ($P < 0.05$ at 300 μ*M*).

Cell cycle arrest by dopa and DHBA

Preliminary temporal and dose-response studies of the cell cycle with DNA flow cytometry indicated that major effects were observable after one cell doubling period (24 h) and with doses of 1–5 m*M*, which were moderately toxic at the cell density required for analysis. At a concentration of 1 m*M* DHBA had a greater effect than dopa, causing accumulation of cells in S phase (Table 4). Both drugs induced arrest in G_1 when used at 5 m*M*.

Discussion

This study showed that in human melanoma cell lines, DHBA closely resembled dopa in toxicity and selectivity in a manner which did not correlate with various markers of melanogenic activity. It is unlikely that the more effective inhibition of DNA synthesis by DHBA compared with dopa is due to more template damage, as detected by direct inactivation of adenovirus [18], and it is therefore assumed to result from an epigenetic effect of the drug. The enhanced incorporation of thymidine 6 h after DHBA treatment is similar to that induced in these lines by sublethal concentrations of the ribonucleotide reductase inhibitor hydroxyurea (P.C. Parsons, unpublished work), an effect which is presumably due to decreased de novo synthesis of deoxynucleotides and consequent increased incorporation of exogenous labelled deoxynucleoside. The action of DHBA over and above that of dopa could therefore be interpreted in terms of a greater effect on DNA polymerase and/or ribonucleotide reductase. The effects of catechols resemble those of 4-hydroxyanisole, an antimelanoma agent that inhibits DNA synthesis and blocks cells in G_1 [5]. The cell cycle study produced the new finding that, like other DNA synthesis inhibitors such as methotrexate [28], the stage of cell cycle arrest by catechols in human cells was dose-dependent. This suggests two types of drug action, one which inhibits entry into G_2/M and a second effect at higher doses, which strongly inhibits DNA synthesis with consequent blocking in G_1. Inhibition of DNA synthesis per se does not appear to explain the selective toxicity of these drugs, because no cross-sensitivity to hydroxyurea or methotrexate was found.

The overall mechanism of toxicity of these agents remains undefined. The significance of oxygen radicals being generated outside the cell in the initial stages [18] was reinforced in the present study by the observation that compounds such as methaemoglobin and pyruvate, which react with hydrogen peroxide [26], markedly reduced toxicity. The inhibition of dopa toxicity by methaemoglobin was of interest for several reasons. First, the varying levels of methaemoglobin in fetal calf serum mean that all experiments in a series should be carried out using the same batch of serum. Second, the possibility of methaemoglobin being formed by these agents in vivo [17] may reduce their potency. It should also be noted that the toxicity of redox-active compounds or their decomposition products need not depend on the production of free radicals. Catechols may act as radical scavengers to inhibit ribonucleotide reductase [1]; anti-oxidants such as butylated hydroxyanisole were active against the mouse B16 melanoma in vivo [10]; and a copper complex with superoxide dismutase-like activity retarded the growth of a rodent carcinoma in vivo [13]. However, the favourable results obtained with redox-active agents in rodent models may not be applicable to humans, because rodent tumours are generally deficient in the Mn form of superoxide dismutase compared with human tumours [13, 29].

Despite variation between cell lines in the phenylalanine and tyrosine pools, the results were of interest because the heavily melanised line MM418 had a much higher phe/tyr ratio than be other lines. The phenylalanine levels apparently were not elevated in MM418, which is consistent with the result of a previous comparison of melanotic and amelanotic mouse B16 melanoma cells [25]; tyrosine levels in the mouse cells were not reported. The present results therefore suggest that tyrosine is depleted in melanotic cells and possibly is a rate-limiting factor in human melanogenesis. In due course it will be of interest to test this possibility in suitably matched cell lines.

The similar incorporation of dopa and tyrosine by the melanised line MM96E, compared with MM96L and He-

La, indicated that melanin synthesis did not consume a significant proportion of these amino acids. Dopa appeared to be incorporated more rapidly than tyrosine, possibly because of covalent binding of the quinone form to cellular targets as proposed for catechols [6]. The results with the tyrosinase MoAb provided independent evidence that neither early nor late stages of the melanisation pathway are implicated in the selective action of catechols against particular melanoma cell lines. The retinoblastoma line was highly sensitive to killing by dopa and DHBA; and catechols were reported to have activity against neuroblastoma [31]. It is therefore possible that neural crest tumours as a group are predisposed to redox sensitivity. Since sensitivity to catechols was expressed in cell lines as the absence of a shoulder on the survival curve, it is conceivable that redox sensitivity results from loss of ability to scavenge radicals being generated from endogenous or exogenous sources. Future studies will therefore be made of glutathione metabolism, a system which provides protection from radicals, is indirectly involved in the melanin biosynthetic pathway, and which may provide cofactor for ribonucleotide reductase [16].

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