INDUCTION OF ERYTHROID DIFFERENTIATION IN MURINE ERYTHROLEUKEMIC CELLS BY SHORT CHAIN ALIPHATIC CARBONYL COMPOUNDS AND THEIR CORRESPONDING PRECURSORS

Evidence for a common inducing signal

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1. Introduction

Proerythroblastoid murine erythroleukemic (MEL) cells may be induced to differentiate along the erythroid pathway by a variety of chemical agents [1–5]. The state of erythroid differentiation is recognized unequivocally by increased hemoglobin synthesis and concomitant morphological changes [5,6]. While the precise nature or the cellular receptor sites of the erythroid differentiation induction signal remain undetermined [1,3], it has been suggested that the cell membrane may be the initiation site [1,3] as evidenced by changes of certain cell membrane-related functions [7,8].

We have shown recently that SeO_2 and H_2SeO_3 trigger erythroid differentiation of MEL cells [3] and suggested further, that possible oxidation by SeO_2 of endogenous membrane carbonyls into dicarbonyls may have initiated erythroid differentiation of MEL cells. If endogenous carbonyls are triggering differentiation, then it may be possible that selected exogenous aliphatic carbonyl compounds may likewise initiate erythroid differentiation of MEL cells. Moreover, any precursor which can be metabolized to its corresponding carbonyl derivative should also act as an inducer or erythroid differentiation.

2. Materials and methods

Murine erythroleukemic (MEL) cells, derived from clone 745, were grown in RPMI 1640 medium containing 10% (v/v) heat-inactivated fetal calf serum and supplemented with glutamine and antibiotics [5]. MEL cells were diluted to 10^5 cells/ml and the compounds listed in table 1 were added 4 h later. The concentration of liquid inducers was determined from their densities. The salts of monocarboxylic acids were dissolved in 10.0 mM Tris–0.15 M NaCl (pH 7.4). Butyraldehyde was diluted with methanol. Since methanol at high concentration (>1000 mM) induces differentiation of MEL cells, the final methanol concentration in the medium was only 1/12th of the level required to elicit differentiation. The cells were harvested at the end of a 5-day incubation period and their viability determined by trypan blue exclusion. Duplicate aliquots containing 2×10^6 total cells were then centrifuged and the hemoglobin concentration was measured by a colorimetric benzidine assay [5].

3. Results and discussion

The comparative capacity of alcohols, aldehydes, ketones and carboxylic acids to induce hemoglobin synthesis in MEL cells is presented in table 1. The data show that hemoglobin synthesis can be induced in MEL cells by 1–5 carbon compounds of various oxidation states with alcohol compounds being less potent inducers than acids, aldehydes, and ketones. Aldehydes were more potent inducers than ketones. This difference may not be ascribed to hydration, since both aldehydes and ketones are readily hydrated

Table 1
Comparative inducing capacity of aliphatic carbonyls and their precursors

Inducer (no. expt.)	Mol. wt.	Conc. (mM)	Average cell no. × 10^6/ml	No. inducer molecules × 10^{23}	μg Hemoglobin/ × 10^6 cells	Hemoglobin/ inducer ratio
Alcohols						
Methanol (7)	32.04	1190	2.0	7.16	1.56 ± 0.2	0.21
Ethanol (4)	46.07	550	1.6	3.31	0.90 ± 0.18	0.26
Ethylene glycol (5)	62.07	545	1.8	3.28	0.89 ± 0.22	0.27
Propanol (9)	60.11	177	1.5	1.06	0.60 ± 0.09	0.56
Glycerol (6)	92.11	720	1.2	4.33	0.72 ± 0.12	0.16
Butanol (6)	74.12	101	1.3	0.61	0.84 ± 0.06	1.4
Aldehydes						
Formaldehyde (9)	30.03	0.009	0.5	0.00005	0.38 ± 0.06	7.6×10^3
Acetaldehyde (8)	44.05	21	1.0	0.12	0.47 ± 0.11	3.9
Butyraldehyde (5)	72.11	0.7	0.4	0.004	1.24 ± 0.17	310
Ketones						
Acetone (7)	58.68	330	2.2	1.98	1.41 ± 0.15	0.71
Butanone (6)	72.12	86	2.6	0.51	1.48 ± 0.25	2.9
2.4-Pentanedione (5)	100.13	28	0.2	0.16	0.60 ± 0.11	3.75
Acids						
Na Acetate (6)	83.05	60	2.1	0.361	0.72 ± 0.07	1.99
Na Propionate (8)	97.08	2.5	3.2	0.015	1.32 ± 0.14	88
Na Butyrate (4)	111.12	1.0	4.3	0.006	1.10 ± 0.27	183

Inducers of hemoglobin synthesis are divided into three functional classes, i.e., alcohols, carbonyls (aldehydes and ketones) and acids. The compounds in each class are listed in the order of increasing chainlength. The number in parenthesis beside the inducer indicates the number of experiments. Molecular weights of inducers are listed in column 2 while the mean concentration range required to induce hemoglobin synthesis is given in column 3. Column 4 lists the average number of total cells determined at the end of each experiment. The mean number of inducing molecules, i.e., the product of inducer concentration times Avogadro's number ($A_N = 6.02 \times 10^{23}$) is listed in column 5. Concentration of hemoglobin in μg ± SEM/10^6 cells after 5 days of incubation as determined by benzidine assay [5] is listed in column 6. The capacity of an inducer to trigger hemoglobin synthesis is defined as the ratio of hemoglobin in μg/10^6 cells divided by the multiples of Avogadro's number of an inducer. Since the concentration of cell inducers is given in the same order of magnitude namely as $N \times 10^{23}$, where N is the multiple of A_N, the ratios listed in column 7 are μg hemoglobin/N with 10^{23} omitted. The average hemoglobin level in non-induced MEL cells was 0.12 ± 0.02 μg/ml with an average yield of 3.8×10^6 cells after 5 days incubation

[9]. Therefore, the nature of the induction initiated by aldehydes as compared to ketones, remains unclear. The presence in molecules of 1–3 hydroxyl groups which could be converted to carbonyl groups showed no inducing effect beyond that of a single hydroxyl group, as evidenced by the responses elicited in MEL cells by ethylene glycol and glycerol. Since carbonyl groups appear to be the most potent inducers, it is possible that the inducing capacity of alcohol compounds proceeds via enzymatic conversion to the corresponding carbonyl derivatives. Alcohol dehydrogenases and ubiquitous enolases present in all cells may provide the requisite mechanism for the conversion of mono- and polyhydroxy alcohols to their respective carbonyl derivatives [10,11]. Similarly, aliphatic acids can be converted to carbonyl derivatives by means of β-oxidation as seen with butyric acid, which is readily catabolized by conversion to the ketone acetoacetate [12]. It should be noted, however, that propionate is catabolized by a different pathway from butyrate [13] and has been shown to be converted to heme by human bone marrow cells [14]. This utilization of propionate by MEL cells does not, however, exclude the existence of an activation signal possibly provided by a carbonyl intermediate such as acetoacetate, pyruvate, or malonic semi-aldehyde [13].

In the past, attempts have been made to correlate the inducing capacity with structural configuration of numerous compounds [1]. It appears that increasing

chain length of active compounds to 4 carbons increases induction of hemoglobin synthesis in MEL cells. The only exception is formaldehyde, an aldehyde of well-known anomalous properties [15]. However, structural configurations of the inducing agent appear to be less important than the presence of a carbonyl group which may provide the initial differentiation stimulus. This conclusion, however, does not negate the possible existence of other differentiation-inducing mechanisms.

The chemical nature of exogenous carbonyl initiators of erythroid differentiation is strikingly similar to the activation of lymphocytes effected by cell membrane carbonyls generated in situ by periodate oxidation of target cells [16–18]. Likewise, the similarity of the inducing mechanism for terminal differentiation of human myeloid and MEL cells has been noted [19]. The fact that carbonyl compounds induce erythroid differentiation of MEL cells and the blastogenic transformation of lymphocytes clearly implies the existence of at least one common initiation signal for these two cell types.

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