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Modifications of Oxido-reductase Activities in Adriamycin-resistant Leukaemia K562 Cells

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ABSTRACT. Adriamycin (ADR), a well-known antitumoral drug, interacts with DNA (nuclear and mitochondrial) and cardiolipin. Moreover, ADR induces numerous mitochondrial modifications in sensitive cells. However, no results have yet been obtained as to the repercussions of drug effects on oxido-reductase activities in ADR-resistant cells. To analyze mitochondrial damage induced by ADR treatment, we investigated lactate content, oxygen consumption, respiratory chain activities, and cytochrome content in ADR-sensitive K562 cells and two ADR-resistant variants (K562/R0.2 and K562/R0.5 cells). Biochemical investigations in ADR-resistant cells showed several mitochondrial modifications (in comparison to the parental cell line) according to the variant line and the physiologic state. More particularly, in K562/R0.5 cells cytochrome c (cyt c) oxidase (COX; EC 1.9.3.1) activity and cytochrome aa3 content dramatically decreased since cells enter into the stationary phase. Regardless of the number of multidrug-resistant cell subcultures in ADR-free medium, the cytochrome c oxidase activity in the stationary phase remained unchanged, indicating an irreversible effect of the drug. These alterations could correspond to several modifications of the nuclear and/or mitochondrial genome(s) following acquisition of the ADR resistance phenotype by K562 cells. BIOCHEM PHARMACOL **56**;4: 451–457, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. adriamycin; cytochrome c oxidase; mitochondria; multidrug resistance; respiratory chain; succinate ubiquinol oxido-reductase.

Among antineoplasic agents used in chemotherapy, ADR§ is an anthracycline which binds to DNA [1] and induces DNA single-strand breaks. Although most ADR taken up by cells can be recovered from the nucleus, a low quantity of drug was located in mitochondria [2] and may modify organelle function through cardiolipin (a respiratory chain effector) and the proteins encoded by the mitochondrial DNA [3]. In addition, it has been suggested that COX (EC 1.9.3.1) is a direct target for ADR [4] and is inhibited by the ferric-adriamycin complex itself [5]. Thus, ADR greatly modifies the structural and functional integrity of the mitochondria in sensitive cells [6].

Conversely, the treatments of tumor cells by heterocyclic cytotoxic drugs [7], such as ADR, can lead to the MDR phenomenon. Among the effects induced by ADR, numerous mutations and partial deletions in DNA have been observed in MDR cells [8]. Intracellular resistance was positively correlated with amplification of multidrug resistance gene (*mdr1*), resulting in the overexpression of the transmembrane P-glycoprotein 170 [9]. This P-glycoprotein, an ATP-dependent drug pump efflux, is implicated in cellular detoxification processes [10]. Moreover, it has been shown that MDR cells have a high glycolytic activity [11]. Nevertheless, it has also been proposed that the metabolic changes may be unrelated to MDR phenotype but induced by drug effect in cells [12]. These results suggest that the cellular bioenergetic is modified in resistant cells.

However, no investigation has yet been conducted on respiratory chain enzyme changes induced by treatment in MDR cells. In a preliminary study, the lactate content of ADR-sensitive and -resistant K562 cell lines was evaluated during cell growth. Oxygen consumption and several respiratory chain complexes were studied, and cytochromes in isolated mitochondria from sensitive and resistant K562 cells were then examined. COX activity in ADR-resistant cells was also evaluated according to the passage number in drug-free medium.

MATERIALS AND METHODS

Chemicals

ADR formulated for clinical use was employed for the culture of cells, and was a gift from Prof. J. Robert (Institut Bergonié). All other chemicals for polarographic and spec-

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§ *Abbreviations*: ADR, adriamycin; COX, cytochrome c oxidase; cyt c, cytochrome c; and MDR, multidrug resistance.

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trophotometric studies of respiratory chain enzymes were supplied by Sigma Chemical Co.

Cell Lines

ADR-sensitive and -resistant K562 cells were kindly provided by Prof. J. Robert. Cells (seeded at 2.5×10^5/mL) were grown in suspension in RPMI 1640 medium containing 10% (v/v) heat-inactivated fetal calf serum (Boehringer Mannheim) and 2 mM L-glutamine (Life Biotechnology). Cells were incubated at 37° in a humidified atmosphere of 5% CO_2-air. The resistant cell lines were obtained by addition to the culture medium of: 0.2 μg/mL of ADR for K562/R0.2 from the parental sensitive cells (K562/S); and 0.5 μg/mL of ADR for K562/R0.5 cells from K562/R0.2 resistant cells. In all experiments, the cells were not exposed to the drug for at least 18 days prior to experimental procedures. For the assays, cells were harvested in an exponential growth phase and in a stationary phase after 48 and 96 hr of culture, respectively. The cellular density was measured by hemocytometer and viability was assessed by the trypan blue exclusion test.

Lactate Measurement

One milliliter of K562 cell cultures was harvested at various times during growth, washed with ice-cold PBS and then incubated in the presence of 1 mL of 0.4 M perchloric acid for 20 min at 4°. After centrifugation at 15,000 *g* at 4° for 5 min, the supernatant was neutralized with potassium hydroxide and stored at −20° until analysis. Lactate content was measured by spectrophotometric determination of NADH formation at 340 nm, pH 9.5 in the presence of lactate dehydrogenase and excess NAD^+, using a lactic acid assay kit (Boehringer Mannheim). The number of cells in each assay was determined and the lactate content expressed as μmol of lactate per 10^6 cells.

Oxygen Consumption

Oxygen consumption rates were determined with a Clark oxygen electrode calibrated between 0 and 100% at 37° with sodium dithionite and atmospheric oxygen (present in the medium). The oxygen concentration in the medium was 240 μM. The cells (3×10^6) were suspended in a total volume of 1 mL of respiratory buffer (0.3 M mannitol, 10 mM KCl, 5 mM $MgCl_2$, 1 mg/mL of BSA, 10 mM KH_2PO_4, pH 7.4) at 37°. After measurement of intact cell respiration, cells were permeabilised with 0.01% (w/v) digitonin. Ten mM succinate, 0.2 mM ATP and 3 μM rotenone (complex I inhibitor) were then introduced into the assay and the oxidation of succinate was measured [13]. Malonate (10 mM) was used to inhibit this oxidation.

Enzyme Assays

Cells in logarithmic or stationary phases were harvested and washed twice in PBS, and stored at −80°. The activities of respiratory chain complexes were spectrophotometrically measured under V_{max} conditions, at pH optima, according to standard procedures [13]. The detailed conditions of enzyme assays have been previously described [13].

The activity of succinate ubiquinol oxido-reductase (EC 1.3.9.9; complex II) was monitored in 10 mM KH_2PO_4 (pH 7.8), 1 mg/mL of BSA, 0.2 mM ATP, 50 μM decylubiquinone, and in the presence of inhibitors, i.e. 3 μM rotenone (complex I), 3 μM antimycin (complex III), and 1 mM KCN (complex IV). The reaction was started by adding 80 μM 2,6-dichlorophenol-indophenol, an artificial electron acceptor. The reduction was measured spectrophotometrically at 600 nm, and results were expressed as nmol 2,6-dichlorophenol-indophenol reduced/min/mg of protein using the extinction coefficient of 19,100 M^{-1}.

The global activity of succinate cyt c reductase activity (complexes II and III) was recorded in 10 mM KH_2PO_4 (pH 7.8) containing 1 mg/mL of BSA, 40 μM oxidized cyt c, 0.2 mM ATP, 10 mM succinate and in the presence of inhibitors, i.e. 3 μM rotenone (complex I) and 1 mM KCN (complex IV). After inhibition of succinate cyt c reductase by 10 mM malonate, 50 μM decylubiquinol was added for the determination of decylubiquinol cyt c oxido-reductase (complex III) activity, which was inhibited by 3 μM antimycin.

COX activity (complex IV) was measured in 10 mM KH_2PO_4 (pH 6.5) containing 1 mg/mL of BSA, 2.5 mM lauryl-maltoside and 10 μM reduced cyt c. On the other hand, the specificity of the reaction was determined by addition of 1 mM KCN, which inhibited the reaction. Results were expressed as nmol of cyt c oxidized or reduced/min/mg of protein using the extinction coefficient of 18,500 M^{-1} at 550 nm for the reduced cyt c.

Preparation of Mitochondria

Mitochondria were isolated from ADR-sensitive or -resistant K562 cells in the exponential and stationary growth phase, after culture in spinner flasks (1 dm^3) according to Rustin *et al.* [13] and Rickwood *et al.* [14]. Cells were washed twice with medium A containing 100 mM sucrose, 1 mM EGTA, 20 mM, 3-(N-morpholino) propanesulphonic acid at pH 7.4, 1 mg/mL of BSA. Cells were then suspended in 1 mL of medium B (medium A to which were added 10 mM triethanolamine, 5% (v/v) Percoll and 0.1 mg/mL of digitonin) for 3 min at 4°. Cells were then disrupted in a tight-fitting glass-teflon homogenizer using 7 up-and-down strokes at 900 rpm. The homogenate was diluted (1/5) in medium C containing 300 mM sucrose, 1 mM EGTA, 20 mM, 3-(N-morpholino) propanesulphonic acid (pH 7.4), 1 mg/mL of BSA and centrifuged twice at 2,500 *g* for 5 min. The resulting supernatant was further centri-

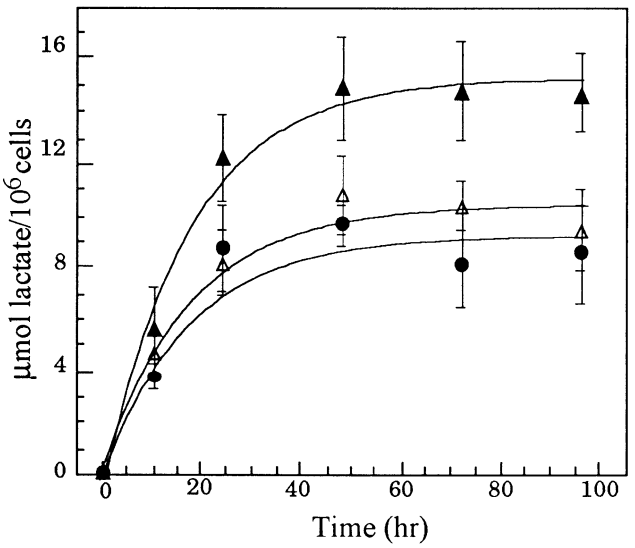

FIG. 1. Lactate content during ADR-sensitive and -resistant K562 cell growth. Cellular lactate was measured with a lactate dehydrogenase assay after extraction, and all three cell lines were seeded at the same density (2.5×10^5 cells/mL) and without ADR as described in Materials and Methods. Data were from three samples of duplicate experiments per cell line. (●) K562/S; (□) K562/R0.2; (▲) K562/R0.5.

fuged at 10,000 *g* for 10 min. The pellet of crude mitochondria was resuspended in medium C. All steps were carried out at 4°.

Analysis of Cytochrome Content

Analysis of cytochrome content was carried out as previously described [14]. Mitochondria (1 mg of protein/mL) were suspended in 300 mM mannitol, 10 mM KCl, 50 mM $MgCl_2$, 10 mM KH_2PO_4 (pH 7.4), containing sodium dithionite to completely reduce the cytochromes, at 25°. The absolute spectrum of oxidized samples was automatically subtracted from the reduced form. Room temperature spectra were recorded with a λ2 spectrophotometer (Perkin-Elmer). The cytochrome content was estimated using the appropriate equations [14].

Protein Content

The protein content was determined by a Biuret procedure using BSA as standard [15].

Statistics

An unpaired Student's *t*-test of the Statview™ software was used for all experiments.

RESULTS

Lactate Production in Sensitive and Resistant K562 Cells

The lactate content was estimated during cell growth (Fig. 1). For each cell line, a plateau was reached at 48 hr after initiation of culture, which corresponded to the exponential growth phase, and the lactate level was maintained until 96 hr. During proliferation, the amount of lactate in the medium was similar for K562/S and K562/R0.2 cells, whereas K562/R0.5 cells showed a significantly ($P < 0.005$) higher lactate level. The lactate content was 14.6 ± 1.5 µmol of lactate/10^6 cells at 96 hr for K562/R0.5 cells and only 8.5 ± 1.9 and 9.4 ± 1.6 for K562/S and K562/R0.2 cells, respectively.

Oxygen Consumption by ADR-sensitive and -resistant K562 Cells According to Physiological State

In exponential growth phases, the oxygen consumption rate (Table 1) with endogenous substrate was similar for all three cell lines. The basal respiratory rate was diminished by 37% for K562/R0.5 cells in stationary phase ($P < 0.02$), whereas the decrease was less marked for parental cells and K562/R0.2 cell lines (22 and 25% respectively, $P < 0.02$). Whatever the physiological state and cell type, endogenous cellular respiration was very sensitive to rotenone (inhibition ca. 90%), the inhibitor of complex I (NADH-ubiquinone oxido-reductase). The addition of an oxidative substrate such as succinate to the respiration medium containing digitonin-permeabilised cells induced a significant stimulation of the oxygen consumption rate. In the expo-

TABLE 1. Oxygen consumption of ADR-sensitive and -resistant K562 cells in logarithmic and stationary growth phase under various conditions

Growth phase	Exponential			Stationary		
Substrate or Inhibitor	K562/S	K562/R0.2	K562/R0.5	K562/S	K562/R0.2	K562/R0.5
None	4.50± 0.39	4.74 ± 0.44	4.22 ± 0.32	3.68 ± 0.35	3.79 ± 0.61	3.07 ± 0.64
Rotenone	0.50 ± 0.10	0.48 ± 0.19	0.54 ± 0.20	0.54 ± 0.22	0.51 ± 0.12	0.47 ± 0.24
Succinate (+ digitonin)	6.08 ± 0.50	8.75 ± 0.79	7.77 ± 0.56	4.49 ± 0.70	6.25 ± 0.32	3.88 ± 0.61
Malonate	0.38 ± 0.08	0.39 ± 0.12	0.41 ± 0.10	0.38 ± 0.08	0.38 ± 0.15	0.40 ± 0.12

Cells were grown and harvested after 48 and 96 hr of culture without ADR. O_2 consumption was measured as described in Materials and Methods. Endogenous respiration (complex I) was inhibited by 3 µM rotenone and malonate, a competitive inhibitor of succinate dehydrogenase, was added at a concentration of 10 mM. Values were expressed in nmol of O_2/min/mg of protein, and were the means of at least five experiments.

TABLE 2. Respiratory chain enzyme activities according to the growth phase of ADR-sensitive and -resistant K562 cells

	A			B		
	Exponential phase			Stationary phase		
Enzyme or Inhibitor	K562/S	K562/R0.2	K562/R0.5	K562/S	K562/R0.2	K562/R0.5
Complex II	8.7 ± 4.8	15.5 ± 2.1	11.8 ± 1.5	10.7 ± 1.4	24.2 ± 5.1	14.5 ± 2.4
Malonate	0.8 ± 0.4	0.9 ± 0.3	1.1 ± 0.5	0.6 ± 0.3	0.9 ± 0.3	0.5 ± 0.2
Complex III	53.3 ± 8.7	39.4 ± 1.8	58.5 ± 11.4	44.5 ± 11.2	52.1 ± 8.8	53.8 ± 11.5
Antimycin	4.1 ± 0.4	5.5 ± 0.9	4.4 ± 1.1	5.2 ± 0.4	4.5 ± 0.3	5.4 ± 2.1
Complex IV	62.4 ± 12.5	69.4± 12.9	67.6 ± 9.9	35.8 ± 6.6	31.9 ± 6.3	6.7 ± 1.9
KCN	0.2 ± 0.05	0.6 ± 0.1	0.1 ± 0.02	0.2 ± 0.1	0.1 ± 0.02	0.1 ± 0.01

The activity of the respiratory chain complex was measured using freeze-thawed cells as described in Materials and Methods. The concentrations were 10 mM malonate (inhibitor of complex II), 3 μM antimycin (inhibitor of complex III), and 1 mM KCN (inhibitor of complex IV), respectively. Each value is given in nmol of substrate oxidised or reduced/min/mg of protein and was the mean of at least seven separate preparations.

nential growth phase, succinate oxidation was higher in MDR cells as compared to parental cells ($P < 0.002$ in both cases). In the stationary phase, the oxygen consumption rate for all three cell lines decreased ($P < 0.01$); however, the loss appeared more marked in the case of K562/R0.5 cells (*ca.* 50%). The addition of malonate, an inhibitor of succinate cyt c reductase, reduced cell respiration by 90% in all cases.

Respiratory Chain Complex Activities of ADR-sensitive and -resistant K562 Cells According to Physiological State

The activity of complex II was significantly higher in K562/R0.2 cells in both the exponential and stationary phases ($P < 0.01$ in both cases) than for K562/S and K562/R0.5 cells (Table 2A-B). Significant enhancement of complex II activity appeared during the stationary phase for the MDR cell lines ($P < 0.05$) and especially for K562/R0.2 cells (*ca.* 50%). On the other hand, for all three cell lines, no significant difference in complex III activity was observed regardless of the growth state of cells. For the three cell lines, complex II and III activities were similarly inhibited (ca. 80%) by malonate and antimycin, respectively.

In the exponential growth phase, complex IV activity was similar for all three cell lines (Table 2A). In contrast, in the stationary phase (96 hr), COX activity diminished by approximately one-half for K562/S and K562/R0.2 cells ($P < 0.002$), and a dramatic reduction of 90% was observed in K562/R0.5 cells ($P < 0.001$). For the three cell lines, complex IV activity was inhibited at least 95% by KCN.

Cytochrome Content in Isolated Mitochondria from ADR-sensitive and -resistant K562 Cells According to Physiological State

The cytochrome spectra of isolated mitochondria from K562 cells (Table 3) were established at room temperature in the presence of dithionite, and thus constituted only an estimation of cytochrome content. In the exponential phase, cyt c content was slightly lower in K562/0.5 as compared to the other cell types, yet cytochrome aa3 and b contents were similar for all three cell lines. In the stationary state, only cytochrome aa3 content was greatly reduced (67%) in K562/0.5 ($P < 0.002$). Thus, cytochrome b/aa3 and c/aa3 ratios were identical in the three cell lines for the exponential phase (*ca.* 1 and 3, respectively) and were very different for K562/0.5 cells in the stationary phase (3.68 and 10.2, respectively).

COX Activity in ADR-resistant Cells According to the Passage Number in Drug-free Medium

We monitored COX activity at 96 hr of culture (contrasts were more pronounced) as a function of the passage number in drug-free medium (Fig. 2) in order to observe a potential progressive restoration of COX activity in K562/R0.5 cells. Cells were washed three times and resuspended in drug-free medium before seeding. COX activity of resistant cells after 10 passages in drug-free medium was identical to that of cells previously treated with 0.2 μg/mL of ADR for K562/R0.2 cells and 0.5 μg/mL of ADR for K562/R0.5 cells.

DISCUSSION

In the past decade, numerous studies have been carried out on the mechanism of MDR of tumor cells to antineoplastic drugs [7, 16] and the effects of ADR [1, 4, 17] on sensitive cells. Among cellular changes associated with ADR resistance, the MDR cells seem to possess a high glycolytic activity [11], which suggests an abnormal mitochondrial bioenergetic. However, no data have been published concerning mitochondrial damage in resistant cell lines consequent to the ADR effects.

We chose to follow certain metabolic parameters (lactate content, respiration, and chain respiratory activities) according to the physiological state because: 1) cells in exponential growth could compensate for partial defects of catalytic subunits of complexes I, II and III (encoded by mitochondrial DNA) by increasing the copy number of the mitochondrial genome; consequently, the modifications could be undetectable in this phase; and 2) cells in the

TABLE 3. Cytochrome content from isolated mitochondria in ADR-sensitive and -resistant K562 cells according to their physiologic state

Growth phase	Exponential			Stationary		
Cells	K562/S	K562/R0.2	K562/0.5	K562/S	K562/R0.2	K562/0.5
Cytochrome b	58 ± 7	60 ± 4	54 ± 8	61 ± 5	72 ± 3	59 ± 4
Cytochrome c	166 ± 27	181 ± 23	135 ± 20	138 ± 18	181 ± 20	161 ± 11
Cytochrome aa3	56 ± 9	61 ± 10	48 ± 6	58 ± 7	56 ± 6	**16 ± 5**
Ratio b/aa3	1.0	1.1	1.1	1.0	1.3	**3.7**
Ratio c/aa3	2.9	2.9	2.8	2.4	3.2	**10.2**

After culture in spinner flasks, cells were harvested at 48 or 96 hr of culture and washed twice in sucrose buffer. The mitochondria were isolated by cell disruption with a tight-fitting glass-teflon homogeniser and sequential centrifugations, and the spectra were achieved as described in Materials and Methods. Values are expressed in pmol cytochromes/mg of mitochondrial protein, and were the means of three experiments.

stationary phase were close to the physiological state of cells *in vivo*.

The lactate content (Fig. 1), an indirect indicator of energetic disorders, remained unchanged in the first resistant cell as compared to parental cell. In contrast, the "anaerobic" glycolysis in K562/R0.5 cells was significantly higher, but this was not due to modifications in growth characteristics. Indeed, the three cell lines showed no significant difference either in doubling times (*ca.* 20 hr) or in cell repartition in the cycle phase (data not shown). On the other hand, lactate content enhancement was generally correlated with the degree of resistance and may reflect increased energy-dependent drug detoxification [11]. It has been shown that glycolysis stimulation can also result from a suitable cellular response resulting from mitochondrial alterations [18]. Consequently, the increased lactate content for K562/R0.5 cells could reflect both an increase in energy requirements and/or organelle defects in the oxidative phosphorylation.

The oxygen consumption rate using endogenous substrates was similar for all three cell lines for each physiological state. Moreover, cellular respiration, using endogenous substrates or succinate, decreased in ADR-sensitive and-resistant K562 cells between the exponential and stationary phases (Table 1). This is in agreement with observations suggesting a relationship between cellular proliferation and mitochondrial functioning [19].

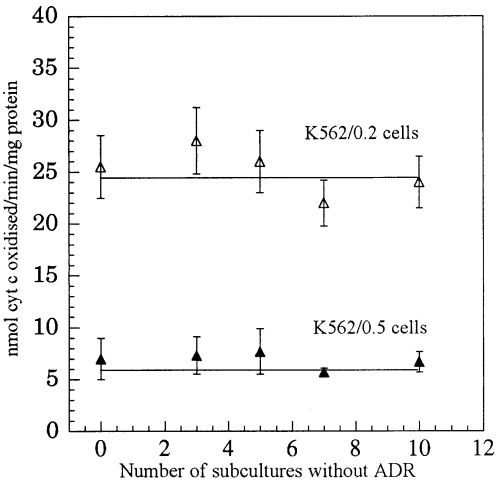

FIG. 2. COX activity of ADR-resistant K562 cells in the stationary phase according to the passage number of cultures without ADR. Enzyme activity was obtained with cells harvested after 96 hr of culture and stored at −80° (□) K562/R0.2; (▲) K562/R0.5.

Enhanced respiration (succinate oxidation) occurred only in K562/R0.2 cells for both physiological states ($P = 0.002$) and was correlated with increased complex II activity. This reflected a strong control of complex II over oxygen consumption. In contrast, complex III activity remained similar for all three cell lines and physiological states.

In the exponential phase, complex IV activity was not significantly modified in MDR phenotype (Table 2 A-B). For all cell lines, the loss of COX activity between 48 and 96 hr was always greater than the decline in the rate of oxygen consumption with succinate. In addition, the alteration of COX activity in K562/R0.5 cells (90%) did not seem to be due to enhanced cell mortality (less than 20% at 96 hr) since the activities of complexes II and III remained unaffected between the two physiological states. However, it has been shown that the respiratory rate is not directly proportional to COX activity and that a minimal activity at this stage can sustain an appreciable flux of respiration and thus ATP synthesis [20]. For instance, in rat muscle mitochondria, it has been determined that an inhibition of COX activity of 50% results in only a 10% loss of the whole respiratory flux and that the drop in COX activity has to exceed the threshold of 70% in order to appreciably decrease the rate of respiration [21]. This threshold in the expression of COX deficiency varies according to the tissue or the cell type; for instance, in rat brain, the threshold up to which there is no noticeable effect of COX inhibition on the respiratory rate is 90% (data not shown).

Cytochromes are constituent molecules of most respiratory chain complexes, and cytochromes a and a3 are implicated in COX structure. The spectrum analyses of cytochromes showed a ratio of cytochrome b/aa3 near 1 in all cases, except for K562/0.5 resistant cells in the stationary phase. The near 1 ratio was in agreement with results in human [13]. The drastic decrease in cytochrome a/a3 content in the stationary phase (67%) for K562/0.5 resistant cells may be correlated to a lower COX activity.

The low COX activity in K562/R0.5 cells in the stationary phase may be due to the ADR in the culture medium. ADR taken up by MDR cells during culture is not fully degraded [22], and could interact with cardiolipin and inhibit complex IV [23]. This hypothesis may be excluded because: 1) the resistant cells were not exposed to ADR for at least 18 days prior to experiments; and 2) in resistant K562 cells, COX activity remained unchanged for ten subcultures in ADR-free culture media (Fig. 2). These data showed that the decrease in COX activity in the stationary phase was not due to a reversible effect of the drug on the resistant cell lines.

The observed decrease in cytochrome c oxidase activity may have been due to a genetic modification which appeared with the acquisition of drug resistance. Indeed, ADR interacts with genomic [1] and/or mitochondrial DNA [3], and generates DNA single breaks, producing numerous mutations such as in the *topoisomerase IIα* gene [24]. Moreover, it was suggested that ADR induces interstrand cross-links at 5′ G-C sequences [25] which often feature 5′ untranslated regions. Thus, the modifications of the nuclear genome leading to ADR resistance could affect the succinate oxido-reductase genes (proteins encoded only by nuclear DNA) and/or their 5′ untranslated regions. Furthermore, in the case of K562/R0.5 cells, the decrease in cytochrome aa3 content and COX activity could be induced by similar mechanisms, but in the latter case the reduction could also be due to the alterations in mitochondrial DNA (for the catalytic subunits).

Finally, ADR-resistant human leukemia K562 cells showed modifications in energy metabolism as compared to the parental cell line: 1) a moderate but significant increase in complex II activity for K562/R0.2 cells; and 2) more pronounced modifications for K562/R0.5 cells, i.e. enhancement of lactate content (anaerobic glycolysis product) which was accompanied in the stationary phase by an irreversible drop in COX activity directly connected to a reduced cytochrome aa3 content. Further studies are necessary to understand the modifications in mitochondrial functions and a possible correlation with mutations and/or deletions of the nuclear and/or mitochondrial genome (s) induced by ADR.

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