Biochemical Pharmacology, Vol. 40, No. 5, pp. 967–973, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

INHIBITION OF PROTEIN SYNTHESIS IN NEOPLASTIC CELLS BY RHEIN

SUSANNA CASTIGLIONE, MARCO G. PAGGI, ANDREA DELPINO,* MASSIMO ZEULI and ARISTIDE FLORIDI†

Laboratory for Cell Metabolism and Pharmacokinetics and *Laboratory of Biophysics, Regina Elena Institute for Cancer Research, Viale Regina Elena 291, 00161, Rome, Italy

(*Received* 23 *October* 1989; *accepted* 26 *March* 1990)

Abstract—The action of rhein, 4,5 dihydroxyanthraquinone-2-carboxylic acid, on protein synthesis of neoplastic cells has been investigated. Rhein decreases amino acid incorporation in all cells tested. The inhibition of incorporation of labeled precursors into acid-insoluble material cannot be ascribed to an impairment of amino acid uptake, which is unaffected by the drug. Tests on cell-free system showed that rhein does not inhibit the TMV-mRNA directed *in vitro* protein synthesis, thus indicating that the protein machinery *per se* is not affected. The inhibition of protein brought about by the drug must be ascribed to an effect on the energy-yielding processes with a remarkable decrease in ATP content. The mechanism is similar to that of other metabolic inhibitors, but rhein, for its capability to inhibit both respiration and glycolysis, is effective at much lower concentrations.

Traditional approaches to the chemotherapy of tumors have relied on the finding that rapidly dividing tumor cells are more susceptible to damage by agents which interfere with nucleic acid metabolism. Nevertheless, rapidly proliferating normal tissues are also damaged by some antineoplastic drugs and such toxicity limits drug utility. A rational alternative approach to cancer chemotherapy would be to identify essential metabolic pathways that contain "regulatory" enzymes of different enzymic composition than normal cells and to target these tumor-specific enzymes for chemotherapeutic inhibition [1].

In the last years several agents have been reported that act through a selective inhibition of tumor energy production [2–6]. One of the most promising compounds is Lonidamine, currently employed as an antineoplastic drug in human patients [7], which acts through a selective inhibition of mitochondrially bound hexokinase [2, 3].

Furthermore, it has been demonstrated that rhein, 4,5 dihydroxyanthraquinone-2-carboxylic acid, an anti-inflammatory drug [8–10], inhibits both respiration and glycolysis of neoplastic cells [11]. The decrease of oxygen uptake is related to the inhibition of electron transport at the dehydrogenase–coenzyme level [12], whereas the reduced lactate production depends on the inhibition of glucose uptake [11].

The observation that rhein inhibits the glycolysis of Ehrlich ascites tumor cells by reducing glucose uptake may be of particular interest considering that some degree of glucose catabolism is required for nucleic acid synthesis and to supply reducing equivalents for the biosynthetic processes [13–15]. Rhein also decreases the growth rate of neoplastic cells *in vitro* (Floridi, manuscript in preparation). Since growth is an energy-requiring process, the ability of rhein to interfere with neoplastic growth strongly suggests that it might affect their synthetic pathways.

The experiments described in this communication were undertaken to evaluate the effect of rhein on protein synthesis in tumor cells. The following questions were addressed: (a) does rhein affect the incorporation *in vitro* of labeled amino acids? (b) does rhein inhibit the uptake of amino acids? (c) is there a relationship between the inhibition of energy metabolism induced by rhein and amino acids incorporation? (d) does rhein inhibit protein synthesis in a cell-free system? Answers to all of these questions are provided below.

MATERIALS AND METHODS

Cells. Ehrlich ascites and Sarcoma 180 tumor cells were grown in 2-month-old male Swiss mice. Klein sarcoma, Yoshida ascites and AS-30D hepatoma tumor cells were grown in C3H mice, male Wistar rats and female Sprague–Dawley rats respectively. The cells were harvested 7 to 10 days after inoculation. The cells were withdrawn from the animals after death and resuspended in a medium containing final concentration of 105 mM NaCl, 5 mM KCl, 50 mM TES,‡ pH 7.40 (NKT). The cells were centrifuged at 2000 *g* at room temperature and washed three times with NKT medium. The packed cells were resuspended at a concentration of 2×10^7 cells/mL. Contamination with other cells did not exceed 0.4% according to differentiated counting of smears stained with the May–Grunwald method. The viability of the cells was routinely 95–98%, as indicated by Trypan Blue exclusion test.

Assay of traslational activity with whole cells. The incorporation of labeled amino acids was performed by incubating 0.2 mL of cellular suspension (4×10^6 cells) in 0.8 mL of NKT medium. The cells were preincubated for 10 min at 30° in a girorotary bath; then 50 μCi of [^{3}H]leucine (sp. act. 120 Ci/mol) was

† To whom correspondence should be addressed.

‡ Abbreviations: TES, *N*-tris[hydroxymethyl]-2-aminoethanesulfonic acid; DNP, 2,4 dinitrophenol; P_i, inorganic phosphate.

added. Glucose, when present, was 10 mM final concentration. The incubation was allowed to proceed for 60 min; at established intervals, 50 μL were withdrawn and spotted onto Whatman 3MM filter-paper disks. The disks were processed with cold and hot trichloroacetic acid (TCA) according to Mans and Novelli [16]. After drying, the disks were transferred into scintillation vials and counted in a LS-1800 Beckman liquid scintillation spectrometer with Aquassure scintillation liquid (New England Nuclear, Boston, MA, U.S.A.) at an efficiency of 60%. Results are always expressed as cpm incorporated/10^5 cells.

Assay of uptake of labeled amino acids by whole cells. The uptake of labeled precursors and the effect of rhein was evaluated in Ehrlich ascites tumor cells. The cells (4×10^6) were preincubated for 10 min in 0.8 mL of NKT buffer at 30°. Rhein was added to a final concentration of 65 μM. At the end of the preincubation time 50 μCi of [^{3}H]leucine or 2 μCi of [^{14}C]protein hydrolysate (sp. act. 57 mCi/mmol) were added. Glucose, when present, was added together with labeled precursors at a final concentration of 10 mM. The incubation was allowed to proceed for 20 min. The cells were then harvested centrifugation at 2000 *g* at 4°, washed three times with cold NKT medium and then resuspended in this medium. The cells were disrupted by addition of sodium dodecylsulfate (SDS, 1% final concentration) and all acid-insoluble material was precipitated by addition of an equal volume of 25% TCA. After 1 hr at 0°, the material was centrifuged for 15 min at 13,000 *g* in a Beckman J21 centrifuge. An aliquot of supernatant (100 μL) was taken up and assayed for radioactivity in 5 mL of Aquassure liquid scintillation. Results were normalized as cpm/10^5 cells.

Zero-trans influx was measured at 30° using an oil-stop procedure. Cells were suspended in NKT buffer at concentration of 2×10^7 cells/mL and preincubated for 10 min in the presence or in the absence of rhein (65 μM). For transport measurements, 0.25 mL of silicone oil ($d = 1.050$) and 0.2 mL of assay solution containing 12.5 μCi of [^{3}H]leucine were added to a 1.5 mL microfuge tube. At time zero, 0.2 mL of cell suspension was rapidly added to the assay tube which was placed in the microfuge. At indicated time the cells were spun through the oil (14,000 *g*) to terminate uptake by the rapid separation from the extracellular substrate. The supernatant and oil were aspirated and the bottom of the tube with cell pellet was clipped off and placed into 7 mL scintillation vials. The pellet was solubilized with 50 μL of 10% SDS and 0.5 mL 1 M NaOH at 65° for 2 hr. The solution was then neutralized with 0.5 mL 1 M HCl and counted in 4.5 mL of Aquassure. Results were always expressed as cpm/10^5 cells.

Assay of adenine nucleotide concentration. The cells (2×10^7) were incubated in a final volume of 2.0 mL of NKT medium at 30° in a girorotary bath. Rhein and glucose, when present, were added at a final concentration of 70 μM and 10 mM respectively. The incubation was allowed to proceed for 1 hr. At the end of the incubation period the content of the flasks was added to 2.0 mL of ice-cold 0.5 M KOH and immediately deproteinized under vigorous shaking on Vortex. After 5 min standing in ice, 1 mL of ice-cold NKT medium was added and the solution transferred in a Centricon 30 (Amicon) and centrifuged for 1 hr at 6000 *g* at 0° in a Beckman J21 centrifuge using JA-20 fixed angle rotor. One mL of the clear ultrafiltered solution was taken up and the pH adjusted to 6.50 by adding 0.3 mL of 1 M KH_2PO_4 solution. The determination of adenine nucleotides (ATP, ADP, AMP) was performed by reverse-phase high-performance liquid chromatography, according to Stocchi *et al.* [18] with an LKB 2150 apparatus equipped with an Erbasil 5 μm C18 column (Carlo Erba, Milan, Italy). The apparatus was connected to an Olivetti M24 computer through a Nelson Analytical series 900 interface. The analysis of chromatograms, as well as the integration of peak areas, was performed with a Nelson Analytical Chromatographic Software, version 3.6. Quantitative measurements were carried out by injection of standard solutions of known concentration.

Assay of traslational activity in vitro. To test the effect of rhein on a cell-free protein synthesis system, a nuclease-treated messenger-dependent rabbit reticulocyte lysate was employed [17]. The incubation mixture contained in a final volume of 25 μL; 20 μL of nuclease-treated rabbit reticulocyte lysate, 3.5 μL of L-[^{35}S]methionine corresponding to 37.5 μCi, and 1.5 μL of tobacco mosaic virus (TMV)-mRNA. Rhein at a final concentration of 70 μM was added in a 1 μL volume. Incubations were carried out at 30°. [^{35}S]Methionine incorporation was assayed as alkali-stable, acid insoluble radioactivity in 2 μL samples.

Chemicals. [^{3}H]Leucine, [^{14}C]protein hydrolysate, L-[^{35}S]methionine, rabbit reticulocyte lysate (nuclease-treated, messenger-dependent) and TMV-mRNA were obtained from the Radiochemical Centre (Amersham, U.K.); TES was obtained from the Sigma Chemical Co. (St Louis, MO, U.S.A.); ATP, ADP, AMP, NAD, NADH, NADP, NADPH were purchased from Boehringer Mannheim, GmbH (Mannheim, F.R.G.). All other reagents were of analytical grade and were purchased from BDH Italia (Milan, Italy). Rhein was a gift from Dr Vittorio Behar, Proter Laboratories, Opera, Milan, Italy.

RESULTS

Effect of rhein on the in vitro *incorporation of amino acids by several tumor cell lines*

Table 1 shows the effect of rhein, both in the presence and in the absence of glucose, on the incorporation of [^{3}H]leucine into TCA-insoluble material by several ascites tumors.

When the medium is supplemented with glucose, the radioactivity in the acid-insoluble material is remarkably higher only in the Yoshida ascites tumor, whereas it is lower in the Ehrlich and sarcoma 180 tumor cells. The addition of rhein decreases the amino acid incorporation in all the cells tested, although the extent of inhibition depends on cell type. AS-30D cells are the most sensitive, while Yoshida ascites and Klein sarcoma cells are the less responsive.

When the respiration is the only energy-yielding process, i.e. in the absence of glucose, rhein is more effective in inhibiting amino acid incorporation.

Table 1. Effect of rhein on the *in vitro* incorporation of [^{3}H]leucine by neoplastic cells

Cells	Glucose			No glucose		
	Control	Rhein	Δ %	Control	Rhein	Δ %
Ehrlich	61,088 ± 3520	32,240 ± 2524	−46	69,608 ± 3220	30,772 ± 2980	−56
Sarcoma 180	46,887 ± 1325	25,085 ± 824	−40	58,899 ± 2350	12,512 ± 1000	−79
AS-30D	32,342 ± 1800	10,282 ± 875	−68	33,381 ± 2320	9469 ± 500	−72
Yoshida	56,580 ± 2780	48,666 ± 1870	−14	48,320 ± 1980	17,910 ± 2520	−63
Klein	43,544 ± 1850	35,664 ± 982	−18	43,952 ± 1530	30,008 ± 1827	−32

The values are expressed as cpm/10^5 cells. Each value ± SD was averaged from five different experiments performed in duplicate. The final concentration of glucose and rhein was 10 mM and 65 μM respectively.

Table 2. Effect of rhein on the amino acid uptake by Ehrlich ascites tumor cells

Additions	^{3}H-Leucine		^{14}C-Protein hydrolysate	
	No glucose	Glucose	No glucose	Glucose
None	40,579 ± 3520	40,981 ± 4260	9083 ± 1320	8089 ± 1240
Rhein	43,097 ± 2780	46,884 ± 3270	9634 ± 985	9658 ± 850

The values are expressed as cpm/10^5 cells. Each value ± SD was averaged from five different experiments performed in duplicate. The final concentration of glucose and rhein was 10 mM and 65 μM respectively.

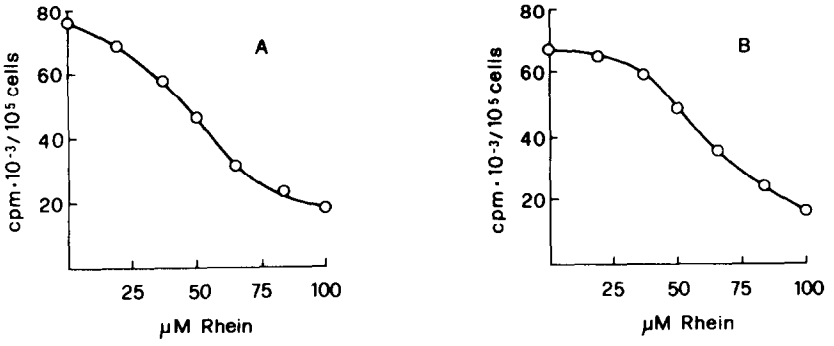

Fig. 1. Dependence of the rate of incorporation of [^{3}H]leucine by Ehrlich ascites tumor cells on rhein concentration in the absence (A) and in the presence of 10 mM glucose (B). Each point was averaged from five different experiments performed in duplicate and yielded reproducible results (±6%).

Klein sarcoma cells are again the less sensitive to the drug.

Effect of rhein concentration on amino acid incorporation

The data reported above clearly demonstrate that amino acids incorporation is strongly inhibited by rhein. To establish the mechanism of action of the drug, systematic investigations were carried out on Ehrlich ascites tumor cells. The choice of this experimental tumor was mainly due to the fact that it was already employed to study the effect of rhein on tumor energy metabolism [11].

Figure 1 shows the incorporation of [^{3}H]leucine as a function of rhein concentration in the presence and in the absence of glucose. In the cells incubated without glucose (A), the incorporation of labeled compound decreases in a sigmoidal manner; half-maximal inhibition occurs at 44 μM and the maximal inhibition is reached at 100 μM rhein. The presence of 10 mM glucose in the incubation medium (B) does not affect the response at highest rhein concentration, whereas low drug concentrations are less effective. This difference lies in the fact that low concentrations of rhein do not inhibit to a considerable extent the glycolysis. Concentrations of rhein up to 37.5 μM do not decrease the incorporation of labeled amino acid into acid-insoluble material. Half-maximal inhibition is reached at 56 μM rhein.

Effect of rhein on the kinetics of incorporation and on the uptake of amino acids

Figure 2 shows the effect of rhein on the rate of protein synthesis in intact cells in the absence (A) and in the presence (B) of glucose. Without glucose, the control incorporates labeled [^{3}H]leucine with an

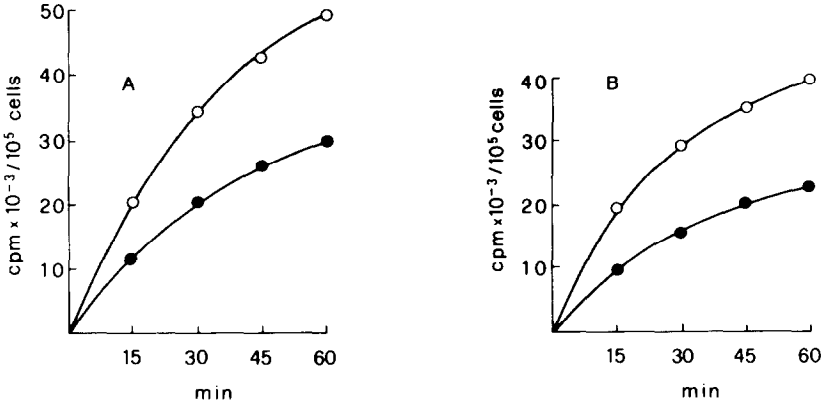

Fig. 2. Kinetics of incorporation of [^{3}H]leucine by Ehrlich ascites tumor cells in the absence (A) and in the presence of 10 mM glucose (B). (○) Control; (●) 65 μM rhein. Eqch point was averaged from five different experiments and yielded reproducible results (±5%).

hyperbolic kinetics; the incorporation is linear during the first 30 min. Subsequently, the rate decreases and approaches to plateau. Rhein, at a final concentration of 65 μM, determines a marked decrease in the rate of incorporation, but the kinetics is similar to that observed for the control. In the presence of glucose (B), the kinetics of incorporation is similar to that found in its absence, but the inhibition achieved by rhein is slightly lower.

The reduced rate of incorporation brougmt about by rhein cannot be explained on the basis of the lack of precursors and cofactors, but must depend on a severe impairment of some factors which control the rate of protein synthesis. There is a well-recognized correlation between concentrative uptake of amino acids and the availability of chemical energy [19]. All compounds that inhibit either respiration or glycolysis in tumors may cause a decrease in the uptake of amino acids. Such inhibitors include, among others, dicumarol [20], lipotropic agents [21, 22] and specific antisera [23]. In view of the ability of rhein to interfere with respiration and glycolysis of neoplastic cells [11], the possibility that the inhibition of protein synthesis could be ascribed to a decreased amino acids uptake was tested. Data on the effect of rhein on amino acids uptake by Ehrlich ascites tumor cells are reported in Table 2. Rhein, both in the absence and in the presence of glucose, does not inhibit the uptake of [^{3}H[leucine and [^{14}C]protein hydrolysate. The inability of rhein to interfere with amino acid transport is further confirmed by the data on zero-trans influx (Fig. 3). "Zero-trans" denotes the transport of a substrate from one side of the membrane to the other side of the membrane where its concentration is initially zero. The amino acid is quickly transported through the cell membrane with an hyperbolic kinetics and the plateau is reached after 15 sec. The addition of 65 μM rhein does not affect neither the time course nor the extent of the influx. In fact, the values obtained in the presence of rhein overlap those of the control.

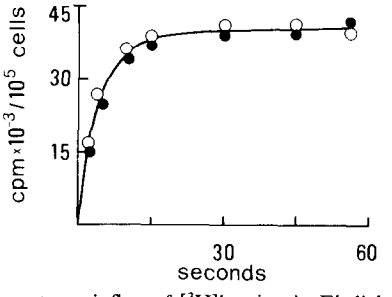

Fig. 3. Zero-trans influx of [^{3}H]leucine in Ehrlich ascites tumor cells. The cells were resupended in NKT medium and pre-incubated for 15 min without (●) or with (○) 65 μM rhein. Cells were mixed over an underlying layer of oil with radiolabelled leucine. Uptake was terminate, at indicated time, by centrifugation through oil. Each point performed in duplicate, was averaged from five different experiments and yielded reproducible results (±6%).

Effect of rhein on the adenylate pool, adenylate energy charge and on protein synthesis in a cell-free system

The data reported above demonstrate that the decrease in protein synthesis brought about by rhein cannot be ascribed to a reduced amino acids uptake. Therefore, the hypothesis that the inhibition of amino acids incorporation could be related to the improvement of energy-yielding processes, was tested and the effect of rhein on adenylate pool and adenylate energy charge was investigated.

Figure 4 shows a typical reverse-phase determination of adenine nucleotides content in Ehrlich ascites tumor cells incubated 1 hr without glucose in the absence (A) and in the presence (B) of 70 μM rhein. In the control the greatest part of adenine nucleotides is present as ATP (82.3%), whereas ADP and AMP are 9.9 and 0.64% respectively. The incubation of the cells with rhein strongly reduces the ATP content (20.8%) and, conversely, raises the

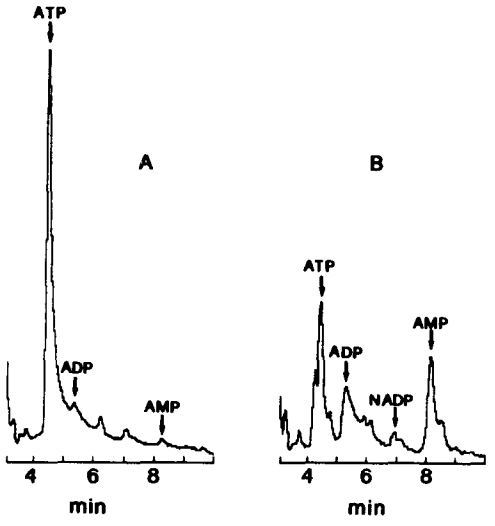

Fig. 4. Representative reverse-phase determination of adenine nucleotides in Ehrlich ascites tumor cells incubated without (A) and with 70 μM rhein (B). The experiments were repeated with four different cell preparations and give reproducible results (±4%).

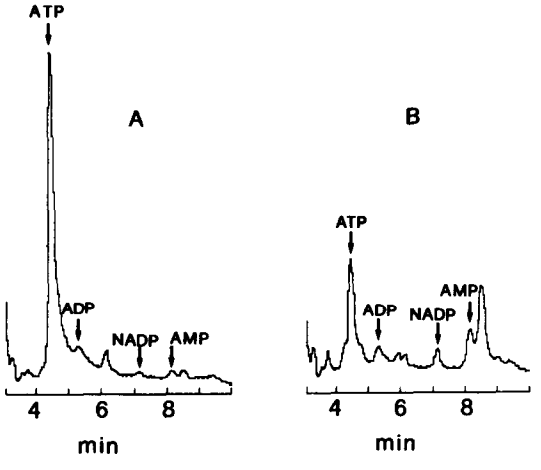

Fig. 5. Representative reverse-phase determination of adenine nucleotides in Ehrlich ascites tumor cells incubated *in the presence of 10 mM glucose*. (A) Control; (B) 70 μM rhein. The experiments were repeated with five different cell preparations and give reproducible results (±3%).

concentration of ADP (20.8%), AMP (19.2%) and $NADP^+$. When the medium was supplemented with 10 mM glucose (Fig. 5) the chromatographic pattern is quite similar to that observed without glucose both in the absence (A) and in the presence (B) of rhein.

On the basis of the data obtained by a quantitative analysis of the chromatograms, the effect of rhein on the adenylate energy charge [24] was evaluated and the values are shown in Table 3. Control cells, both in the absence and in the presence of glucose, appear to maintain the adenylate energy charge at a value of approximately 0.90, thus indicating that the ATP-regenerating reactions are activated. In the

Table 3. Effect of rhein on the adenylate energy charge in Ehrlich ascites tumor cells

	Adenylate energy charge			
	No glucose	Δ %	Glucose	Δ %
Control	0.94 ± 0.04		0.92 ± 0.02	
Rhein	0.51 ± 0.03	−46	0.62 ± 0.01	−33

Each value ± SD was averaged from four different experiments performed in duplicate. The final concentration of glucose and rhein was 10 mM and 70 μM respectively.

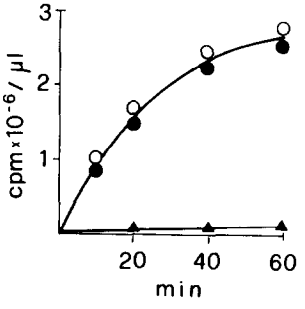

Fig. 6. Effect of rhein on cell-free protein synthesis with a nuclease-treated TMV-mRNA directed rabbit reticulocyte lysate. (○) Control; (●) 70 μM rhein; (▲) no TMV-mRNA. The experoent was repeated three times and gives reproducible results (±8%).

cells incubated with rhein, but without glucose, the value of the adenylate energy charge is lowered to 0.51 (−46%). This effect depends on a decreased ATP synthesis because of the inhibition of the oxidation of substrates entering first two energy-conserving sites of the respiratory chain and of the oxidative phosphorylation [12]. In the presence of glucose, the inhibition of respiration in Ehrlich ascites tumor cells normally does not modify this metabolic parameter [25, 26], but rhein, by inhibiting also the glycolysis [11], lowers the value of the adenylate energy charge to 0.62 (−33%).

Therefore, these data clearly indicate that the inhibition of the incorporation of labeled precursors into acid-insoluble material of neoplastic cells depends on a decreased ATP availability.

Nevertheless, the possibility that rhein might interfere with the synthetic protein machinery *per se* was investigated and Fig. 6 shows its effects in a cell-free system. The rabbit reticulocyte lysate, in the absence of TMV-mRNA, incorporates labeled amino acids to a very low extent. On the contrary, the complete system, i.e. with mRNA, shows a very high rate of incorporation which remains linear for 20 min, then decreases and approaches a plateau level. The addition of 70 μM rhein does not induce any modification either in the rate or in extent of incorporation. In fact, the values obtained with rhein are very close to those of the control and, in any case, not lower enough to justify a specific action of this drug on protein synthetic machinery.

DISCUSSION

The observations recorded in this paper demonstrate that rhein decreases the rate of incorporation of labeled precursors into acid-insoluble material by impairing energy-yielding pathways.

Tests on the effect of rhein on a cell-free system showed that it does not inhibit TMV-mRNA-directed *in vitro* protein synthesis, thus indicating that the protein synthetic machinery is not affected *per se*. The inhibition of incorporation of labeled precursors into TCA insoluble material cannot be ascribed to a decrease of the uptake, which is unaffected by rhein. Although concentrative uptake is an energy-requiring process [27, 28] and compounds that decrease the amount of available ATP should give a simultaneous inhibition of amino acid uptake, the extent of inhibition of the energy-yielding processes not always parallel that of amino acids uptake and incorporation [19. Nevertheless, in spite of the unaffected amino acid uptake, there is a reduced incorporation into TCA insoluble material. Freudenberg and Mager [29] demonstrated that the rate of protein synthesis is correlated with ATP levels and that it is inhibited by the accumulation of the product of ATP splitting, i.e. ADP and AMP, which interfere with the process of peptide chain elongation. This inhibitory effect of ADP and AMP is not detectable under conventional methods of assaying protein synthesis *in vitro* because of their rapid conversion to ATP by the ATP-regenerating system included in the standard reaction mixture. Even though the inhibition of amino acid incorporation can be produced by agents which do not affect ATP, the inhibition is almost inevitable when ATP production is impaired. Therefore, the inhibition of the rate of protein synthesis in intact cells induced by rhein must be ascribed to a decreased ATP availability as a consequence of the reduced rates of respiration and glycolysis. This conclusion is further supported by the observation that the extent to which rhein inhibits the incorporation of amino acids into acid-insoluble material depends on the presence of glucose since, in its absence, rhein is more effective in inhibiting the rate of protein synthesis. The ability of glucose to partially overcome rhein inhibition may be essentially ascribed to two factors.

The first is that the neoplastic cells can utilize equally well the energy from respiration and glycolysis, both anaerobic and aerobic, for thier biosynthetic processes [30]. The uncoupling of oxidative phosphorylation by DNP strongly decreases the incorporation of amino acids into proteins [31]. Nevertheless, the addition of glucose overcomes this inhibition since DNP stimulates the aerobic lactate production through an activation of mitochondrial ATPase which raises intracellular P_i concentration. However, the decrease in respiratory ATP is partially balanced by the increase of the glycolytic ATP, but if the aerobic glycolysis is inhibited the rate of incorporation of labeled precursors is almost completely abolished [31]. Similar results, in a medium supplemented with glucose, were obtained with respiratory inhibitors, such as cyanide, azide, oligomycin etc. [30, 31]. Glucose stimulates protein synthesis through an increased ATP generation with a rise in steady-state levels of this nucleotide. This effect is a widespread phenomenon since it has been observed in a large variety of mammalian cells and tissues [22, 31–35].

The second factor lies in the mechanism of action of rhein. Rhein inhibits respiration, aerobic and anaerobic glycolysis in neoplastic cells [11], but, as lonidamine [4], it needs mitochondria with electron carriers in a relatively oxidized state in order to exert its inhibitory effect on oxidative metabolism. Rhein, in fact, inhibits the oxidation of NAD-linked substrates as well as that of succinate only when it is stimulated by uncouplers or ADP, whereas it is ineffective on state 4 respiration [12]. Glucose, after an initial stimulation (1 min), inhibits the oxygen consumption and induced a more reduced state of cytochrome *b* [36]. Therefore, the low respiratory rate and the more reduced state of the carriers of the first two energy-conserving sites of the respiratory chain make the cells less sensitive to rhein. The effect of rhein on the rate of protein synthesis is similar to that observed for other metabolic inhibitors, but rhein, for its capability to inhibit both respiration and glycolysis, is effective at much lower concentrations.

In conclusion, these data demonstrate that rhein is very effective in reducing the rate of protein synthesis of neoplastic cells *in vitro* by affecting the ATP availability and clearly indicate once more the feasibility of an antineoplastic chemotherapy that is not directed towards nucleic acid synthesis.

Moreover, the observation that rhein lowers the intracellular ATP content could be of considerable importance because it strongly suggests that rhein might be employed as a biochemical modulator [37] to revert or to reduce the multidrug resistance of neoplastic cells by increasing steady-state drug levels through an impairment of the functional properties of 170 kDa P-glycoprotein [38, 39].

Acknowledgements—This work was supported by AIRC/88 and 89. The authors thank Mr Luigi Dall'Oco for his skilful graphic and photographic work.

REFERENCES

1. Hilf R, Murant RS, Narayanan U and Gibson SL, Relationship of mitochondrial function and cellular adenosine triphosphate levels to hematoporphyn derivative-induced photosensitization in R3230AC mammary tumors. *Cancer Res* **46**: 211–217, 1986.
2. Floridi A, Paggi MG, Marcante ML, Silverstrini B, Caputo A and De Martino C, Lonidamine, a selective inhibitor of aerobic glycolysis of murine cancer cells. *J Natl Cancer Inst* **66**: 497–499, 1981.
3. Floridi A, Paggi MG, D'Atri S, De Martino C, Marcante ML, Silvestrini B and Caputo A, Effect of lonidamine on the energy metabolism of Ehrlich ascites tumor cells. *Cancer Res* **41**: 4661–4666, 1981.
4. Floridi A and Lehninger AL, Action of the antitumor and antispermatogenic agent lonidamine on electron transport in Ehrlich ascites tumor mitochondria. *Arch Biochem Biophys* **226**: 73–83, 1983.
5. Suolinna EM, Lang DR and Racker E, Quercetin, an artificial regulator of the high aerobic glycolysis of tumor cells. *J Natl Cancer Inst* **33**: 1515–1519, 1974.
6. Holshouser MH, Loefler LJ and Hall IH, Effects of 3-chloromethylthiocromone-1,1-dioxide on nucleic acid, protein and anaerobic metabolism of Ehrlich ascites tumor cells. *J Pharm Sci* **71**: 857–861, 1982.

7. *Lonidamine, Proceedings of the Second International Symposium* (Eds. Silvestrin B, Caputo A, Band PR and Young CW). *Oncology* **41**: suppl 1, 1–123, 1984.
8. Franchi E, Micheli S, Lovacchi L, Friedmann CA and Zilletti L, The influence of rhein on biosynthesis of prostaglandin-like substances "in vitro". *J Pharm Pharmacol* **35**: 262–264, 1982.
9. Pomarelli P, Berti M, Gatti MT and Mosconi PA, Non-steroidal anti-inflammatory drug that stimulates prostglandin release. *Il Farmaco* **38**: 836–842, 1980.
10. Raimondi L, Bianchelli-Soldaini G, Buffoni F, Ignesti G, Massacesi L, Amaducci L and Friedmann CA, Rhein and derivatives. *In vitro* studies on their capacity to inhibit certain proteases. *Pharmacol Res Commun* **14**: 103–112, 1982.
11. Floridi A, Castiglione S, Bianchi C and Mancini A, Effect of rhein on the glucose metabolism of Ehrlich ascites tumor cells. *Biochem Pharmacol* **40**: 217–222, 1990.
12. Floridi A, Castiglione S and Bianchi C, Sites of inhibition of mitochondrial electron transport by rhein. *Biochem Pharmacol* **38**: 743–751, 1989.
13. Nakashima RA, Scott LJ and Pedersen PL, The role of mitochondrial hexokinase binding in the abnormal energy metabolism of tumor cell lines. *Ann NY Acad Sci* **488**: 438–450, 1986.
14. McKeehan WL, Glycolysis, glutaminolysis and cell proliferation. *Cell Biol Int Rep* **6**: 635–650, 1982.
15. Nakashima RA, Paggi MG and Pedersen PL, Contributions of glycolysis and oxidative phosphorylation to adenosine-5′-trisphosphate production in AS-30D hepatoma cells. *Cancer Res* **44**: 5702–5706, 1984.
16. Mans RJ and Novelli GD, Measurement of incorporation of radioactive amino acids into protein by a filter-paper disk method. *Arch Biochem Biophys* **94**: 48–53, 1961.
17. Pelham HRB and Jackson RJ, An efficient mRNA-dependent translation system for reticulocyte lysates. *Eur J Biochem* **67**: 247–256, 1976.
18. Stocchi V, Cucchiarini L, Magnani M, Chiarantin L, Palma P and Crescentini G. Simultaneous extraction and reverse-phase high-performance liquid chromatographic determination of adenine and pyridine nucleotides in human red blood cells. *Anal Biochem* **146**: 118–124, 1985.
19. Johnstone RM and Scholefield PG, Amino acids transport in tumor cells. *Adv Cancer Res* **9**: 143–226, 1961.
20. Ellis DB and Scholefield PG, The effect of uncoupling agents on the uptake and incorporation of glycine by transplantable tumors. *Cancer Res* **21**: 650–657, 1961.
21. Ayuso-Parrilla MS and Parrilla R, Control of hepatic protein synthesis: differential effect of ATP levels on the initiation and enlongation steps. *Eur J Biochem* **55**: 593–599, 1975.
22. Johnstone RM and Quastel JH, Effect of lipotropic agents on excnange diffusion in Ehrlich ascites carcinoma cells. *Biochim Biophys Acta* **46**: 527–532, 1961.
23. Bickis IJ, Quastel JH and Vas SI, Effect of Ehrlich antisera on the biochemical activities of Ehrlich carcinoma cells *in vitro*. *Cancer Res* **19**: 602–607, 1959.
24. Atkinson DE, The energy charge of adenylate pool as a regulatory parameter: interaction with feedback modifiers. *Biochemistry* **7**: 4030–4034, 1968.
25. Yushok WD, Control mechanism of adenine nucleotide metabolism of ascites tumor cells. *J Biol Chem* **246**: 1607–1617, 1971.
26. Kaminskas E and Nussey AC, Effect of methotrexate and of environmental factors on glycolysis and metabolic energy state in cultured Ehrlich ascites carcinoma cells. *Cancer Res* **38**: 2989–2996, 1978.
27. Christensen HN and Riggs TR, Concentrative uptake of amino acids by the Ehrlich mouse ascites carcinoma cells. *J Biol Chem* **194**: 57–68, 1951.
28. Heinz E and Mariani HA, Concentration work and energy dissipation in active transport of glycine into carcinoma cells. *J Biol Chem* **228**: 97–111, 1957.
29. Freundenberg H and Mager J, Studies on the mechanism of the inhibition of protein synthesis induced by intracellular ATP depletion. *Biochim Biophys Acta* **232**: 537–555, 1971.
30. Aisenberg AC, *The Glycolysis and Respiration of Tumors*, pp. 181–193. Academic Press, New York, 1961.
31. Floridi A, Delpino A, Nista A, Feriozzi R, Marcante ML, Silvestrini B and Caputo A, Effect of lonidamine on protein synthesis in neoplastic cells. *Exp Mol Pathol* **42**: 293–305, 1985.
32. David M and Avi-Dor Y, Stimulation of protein synthesis in cultured heart muscle cells by glucose. *Biochem J* **150**: 405–411, 1971.
33. Jefferson LS, Wolpert EB, Goger KE and Morgan KE, Regulation of protein synthesis in heart muscle. III. Effect of anoxia on protein synthesis. *J Biol Chem* **246**: 2171–2178, 1978.
34. Means AR and Hall PF, Protein biosynthesis in the testis. II. Role of adenosine trisphosphate in stimulation by glucose. *Endocrinology* **83**: 86–89, 1968.
35. Van Venrooig WJW, Henshaw EC and Hirsch CA, Effect of deprival of glucose or individual amino acids in polyribosome distribution and rate of protein synthesis in cultured mammalian cells. *Biochim Biophys Acta* **259**: 127–137, 1972.
36. Chance B and Hess B, Metabolic control mechanism. IV. The effect of glucose upon the steady-state of respiratory enzymes in the ascites cells. *J Biol Chem* **234**: 2421–2427, 1959.
37. Leyland-Jones B and O'Dwyer PJ, Biochemical modulation: application of model laboratory to the clinic. *Cancer Treat Rep* **70**: 219–229, 1986.
38. Beck WT, The cell biology of multiple drug resistance. *Biochem Pharmacol* **36**: 2879–2887, 1987.
39. Bradley G, Juranka PF and Ling V, Mechanism of multidrug resistance. *Biochim Biophys Acta* **948**: 87–128, 1988.