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The effect of AZT and chloroquine on the activities of ricin and a saporin–transferrin chimeric toxin

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Abstract

This study deals with the combination of chloroquine (CQ, an anti-malaric drug) and 3′-azido-3′-deoxythymidine (AZT, anti-human immuno-deficiency virus (HIV) drug) with a chimeric toxin (TS) obtained by chemical linking of saporin (a ribosome inactivating protein from the plant *Saponaria officinalis*) and human transferrin, in the intoxication of the human chronic myeloid leukaemia cells (K562). Our data demonstrate that AZT, at concentrations comparable to those reached in the blood of HIV-infected patients under pharmacological treatment with this drug, can increase the toxicity of TS in cooperation with CQ inducing an increased effect on protein synthesis in K562 cells (~50% inhibition of protein synthesis for TS alone, and TS with AZT and ~70% with both AZT and CQ). Furthermore, pre-treatment of cells with AZT alone can induce an increase of apoptosis in K562 cells intoxicated with TS. By comparing data obtained with the model toxin ricin, we get indications that the two toxins partially differ in their intracellular routes, also suggesting that chimeric constructs containing ricin-like toxins (i.e. immunotoxins) could be coupled with the use of common and cheap drugs for the treatment of cancer in HIV-infected patients.

Keywords: AZT; Chloroquine; Toxins; Transferrin; Ricin; K562 cells

1. Introduction

In the last years the treatment of cancer has been approached by the evolution of the early Erlich's concept of the ''magic bullet'' [1], interpreted as the use of targeted toxins directed towards antigens or receptors over-expressed on the surface of tumor cells. These chimeric molecules have been called immunotoxins [2–4] since their original formulation was that of an antibody linked to bacterial or plant protein toxins. Further molecules have been developed using hormones, cytokines or growth factors as carriers to target cancer cells; great effort has been done in the production of the recent recombinant chimeras [2] that utilize antibody fragments (scFv or dsFv) or cytokines (i.e. interleukin-2 (IL2)) fused to truncated forms of bacterial toxins (i.e. Pseudomonas Exotoxin (PE) from *Pseudomonas aeruginosa* and Diphteria Toxin (DT) from *Corynebacterium dyphteriae*). Some of these molecules have entered clinical trials [5,6] and recently the fusion IL2-DAB38 (a truncated DT derivative) has been approved by the Food and Drugs Administration (FDA) in the USA for commercialization under the name ONTAK. Immunotoxins have been often realized by the use of plant toxins, belonging to the ribosome inactivating proteins (RIPs) [7,8] family, such as ricin and saporin. They are enzymes that catalytically remove a single adenine residue from the ribosomal rRNA (28S or 23S) by the hydrolysis of the *N*-glycosidic bond between the base and the ribose [9,10]. Upon this alteration the elongation factors (i.e. EF2 in eukaryotes) appear unable to tightly bind the ribosome and protein synthesis results interrupted. Ricin (RCA), the most known among plant toxins, is a heterodimeric glycoprotein, composed of two chains (A and B) joined by a disulfide bridge [11]. The B chain is a lectin, whose ability to bind glycolipids and glycoproteins on the cell membrane allows ricin to be easily adsorbed by almost all cell types and then being one of the powerful toxic substances known

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[8]. Saporin instead belongs to the monomeric plant toxin family, being structurally and functionally very similar to ricin A chain (RTA) [12]. In the construction of immunotoxins the B chain of ricin has been substituted with antibodies, via chemical linkage to the A chain of the toxin or by recombinant strategies, but more often monomeric RIPs have been used. Among the large number of targeting molecules, transferrin has been studied as a possible carrier for RIPs [13–17] and recently anti-transferrin receptor antibodies have been used for the production of immunotoxins [18,19] used for intra-tumor administration in brain cancers. Although transferrin-based immunotoxins may be hampered in their use in vivo by the influence of free transferrin and iron saturation level [20], they may be viewed as model chimeras to study cellular absorption [15] and the effect of potentiating substances [16,21].

The deoxynucleoside analog 3′-azido-3′-deoxythymidine (AZT) is an inhibitor of HIV reverse transcriptase still used in therapy of HIV-infected patients [22–24]. It has been reported to have controversial effects on the toxicity induced by protein synthesis inhibitors such as the plant toxins ricin and modeccin or bacterial PE and DT [25–27]. Furthermore, we reported [28] that AZT could influence the intracellular routing of the transferrin receptor in K562 cells, inducing a delay in endocytotic phase of the transferrin-receptor (Tf-TfR) complex.

In this paper we investigated the effects of AZT on the cytotoxicity induced by a chemical conjugate between transferrin and the plant toxin saporin (TS) in K562 cells. Since many HIV-infected patients develop tumors, their systemic treatment with AZT may interfere with the use of immunotoxins based on ricin-like toxins. We demonstrate that AZT (at 40 μM concentration) not only does not inhibit TS cytotoxicity, but even an increase of apoptosis may be induced if K562 cells are previously exposed to the drug.

Furthermore, a comparison between the chimeric toxin and ricin suggests that their intracellular routings partially differ, thus confirming previous results [15]. The simultaneous addition of chloroquine and AZT to the cell suspension enhances TS toxicity, thus suggesting their potential application in therapeutic protocols.

2. Materials and methods

2.1. Synthesis of the conjugate

Saporin was purified from *Saponaria officinalis* seeds as described [29]. The toxin was then chemically conjugated to human holo-transferrin introducing a disulfide bridge by the use of *N*-succimidyl-3-(2-pyridyldithio) propionate (SPDP) and 2-iminothiolane (Sigma–Aldrich, USA), as already described [15].

Ricin was purified from *Ricinus communis* seeds and conjugated to fluorescein isothiocyanate as described [30].

2.2. Cell culture and treatment

K562 human erythroleukemic cells and HSB2 human leukemia T-cells were maintained in exponential growth at 37 °C in a humidified atmosphere supplied with 5% CO_2, in RPMI 1640 culture medium containing 10% heat-inactivated foetal calf serum, 2 mM L-glutamine, 50 U/ml penicillin and 50 μg/ml streptomycin.

Incubations with 40 μM AZT and/or with TS (10^{-12} to 10^{-7} M) have been performed in a 96-well plate at 5×10^5 cells/ml in RPMI 1640 containing 25 mM HEPES, pH 7.4, 0.2% BSA for 1 h at 37 °C; then drug-free complete medium was added to the culture and the incubation extended for further 24 or 48 h. Cell counting and viability were determined at various times by Trypan blue exclusion method.

2.3. Cell proliferation and viability assay

Cytotoxicity has been evaluated by use of the WST1 reagent (Roche Diagnostics) added directly to the cell culture at the end of each incubation period. The absorption at 450 nm, produced by the reduction to formazane salt by NADH-dependent mitochondrial dehydrogenases, was evaluated in a spectrophotometer Perkin Elmer LS-50.

2.4. Protein synthesis inhibition

Upon toxins treatment, 1×10^6 K562 or HSB2 cells were incubated at 37 °C for 30 min in leucine-free RPMI medium, pH 7.2; cells were then washed and 1 ml of medium containing ^{3}H-leucine (1.25 μCi/ml) was added. After incubation for 2 h at 37 °C, the cells were washed twice with cold PBS and lysed with appropriate buffer (1% nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS). After NaOH–H_2O_2 treatment, proteins were precipitated with 25% TCA on glass microfiber filter discs. Radioactivity was measured by a β-counter (Packard tricarb 4000) after dissolving the discs in a suitable scintillation liquid.

Each measure was repeated in triplicate and each experiment was at least in duplicate.

Data are reported as percentage of protein synthesis inhibition, calculated for each experimental point containing toxins with reference to the counts of the relative control sample.

2.5. Determination of apoptosis

Nuclear morphology and cell viability were analysed by double acridine orange (AO) and ethidium bromide (EB) staining. After mixing with an equal volume of a solution containing 100 μg/ml EB and AO, the cell suspension was examined with a fluorescence microscope. Green clumped nuclei indicated chromatin condensation with intact membrane structures (early apoptosis); orange cells with

clumped nuclei indicated later apoptosis. Nuclei of necrotic cells appeared uniformly stained by EB.

Caspase-3 activity has been measured by using the fluorescent substrate Ac-DEVD-AMC. Fluorescence emission was monitored on a Perkin Elmer LS-50B spectrofluorometer, setting excitation at 380 nm and emission at 460 nm.

2.6. Confocal microscopy

K562 cells grown in RPMI medium were incubated at a density of 3×10^5 ml^{-1} with 3×10^{-7} M ricin labeled with fluorescein isothiocyanate as described [30] or with 60 μg/ml transferrin labeled with ALEXA 594 (from Molecular Probes, Oregon, USA), for 30 min at 37 °C in RPMI medium free of transferrin. At the end of the incubation cells were washed with ice-cold HBS (20 mM HEPES, 0.15 M NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.4) and immediately fixed with 4% *para*-formaldehyde in PBS for 10 min at room temperature. The cells were rinsed again extensively with cold PBS, seeded on glass coverslips coated with poly-L-lysine at 25 °C for 30 min. After adhesion to the glass surface, cells were washed three times with PBS and mounted onto object glasses using Mowiol 88 solution (Hoechst). Confocal images were obtained with a Sarastro2000 confocal microscope connected to a Silicon Graphics workstation and analysed with Molecular Dynamics software.

3. Results

3.1. Protein synthesis inhibition

The toxicity of TS conjugate at different concentrations was tested on K562 cells. Preliminary experiments with the colorimetric method (WST1) confirmed previously reported [16] ID_{50} values around 10^{-8} M (data not shown).

Starting from these observations, we studied the effect of 40 μM AZT on the protein synthesis inhibition induced by TS on K562 cells.

AZT, when added together with the toxin to cell suspension, did not change significantly the toxicity of the chimeric toxin (tested both at 10^{-8} and 10^{-7} M, with 52 and 89% inhibition of protein synthesis, respectively) after 48 h incubations, and only slightly inhibited protein synthesis after 24 h incubation (data not shown).

We then tested the effect of AZT used in a pre-treatment protocol on cells comparing TS and ricin (RCA) toxicities. In these experiments AZT was added 24 h before (pre-AZT) and/or at the same time as the toxin (+AZT). As shown in Fig. 1A, TS-induced toxicity was practically unaffected by the presence of AZT, independently on the time of addition of the drug. On the contrary, the toxicity of RCA (10^{-11} M) was significantly attenuated if AZT was added at the same time as the toxin

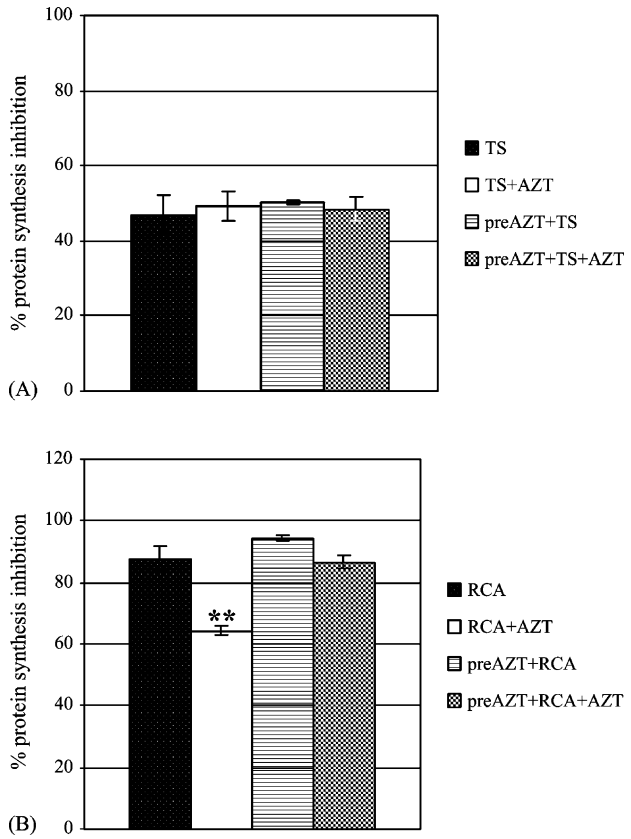

Fig. 1. Protein synthesis in K562 cells measured as incorporation of ^{3}H-leucine after incubation of cells with 10^{-8} M TS or 10^{-11} M RCA for 24 h in the presence or in the absence of 40 μM AZT. K562 cells were also pre-treated with AZT and in one experiment AZT was re-added together with the toxin at the end of the pre-incubation period. Data are reported as percentage of protein synthesis inhibition with respect to control untreated cells. Bars represent S.D. Symbols for single experimental conditions are reported near the plot; ** $p < 0.01$ vs. RCA.

(Fig. 1B), but this effect was abolished if the drug was given 24 h before the cells were exposed to the toxin (pre-AZT).

Fig. 2 shows the effect of a well-known enhancer of chimeric toxins, the drug chloroquine (CQ), on TS and RCA-induced inhibition of protein synthesis. To check CQ and AZT we used cells pre-incubated with 40 μM AZT, since in these conditions RCA toxicity appears to be scarcely influenced by AZT itself.

TS activity was greatly potentiated when CQ was added at 50 μM concentration (data not shown), and the presence of CQ and AZT inhibited almost completely protein synthesis of K562 cells. In these conditions CQ can heterogeneously induce cytotoxicity in the cultured cells, thus hampering an easy interpretation of the results. In view of a possible use of CQ as a potentiating drug "in vivo" and to reduce the related side effects, we assayed CQ at 10 μM. In these conditions CQ is scarcely cytotoxic both alone and in combination with AZT (Fig. 2); CQ was able to enhance TS toxicity (up to 70% protein synthesis inhibition) when used together with AZT.

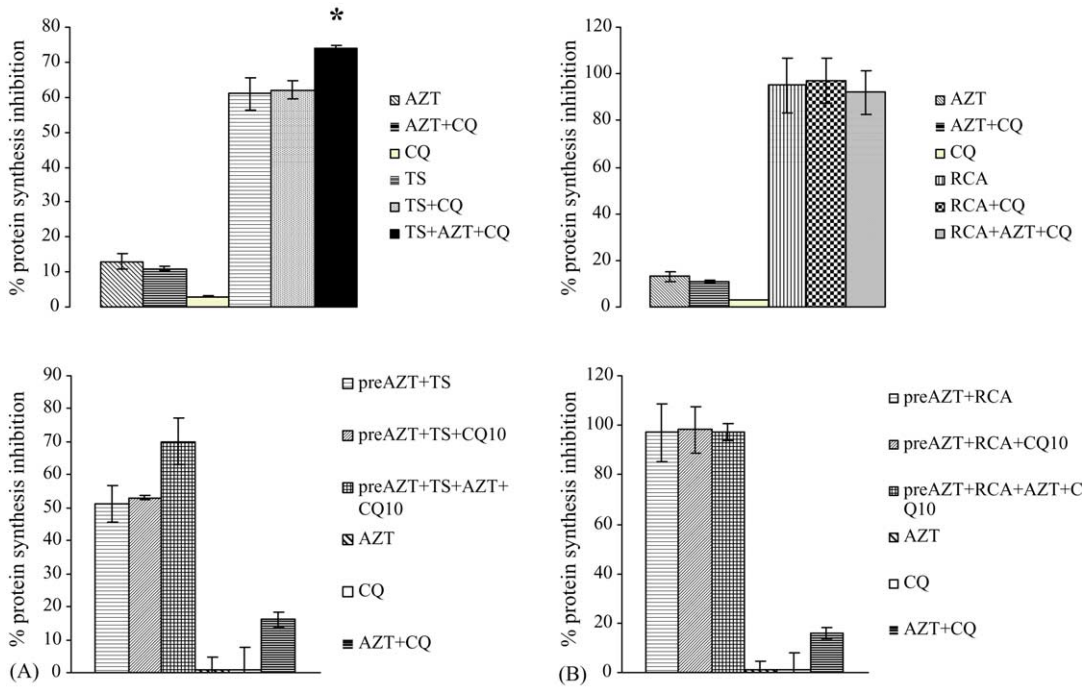

Fig. 2. Protein synthesis in K562 cells measured as incorporation of ^{3}H-leucine after incubation of cells with 10^{-8} M TS or 10^{-11} M RCA for 24 h after a pre-treatment with 40 μM AZT and in the absence or in the presence of 10 μM chloroquine. Data are reported as percentage of protein synthesis inhibition with respect to control untreated cells. Bars represent S.D. Symbols for single experimental conditions are reported near the plot. * $p < 0.05$ vs. pre-AZT + TS.

By contrast, RCA (at 10^{-11} M, Fig. 2B) toxicity was unaffected in these conditions by the presence of both CQ and AZT.

In a previous paper [25] AZT (1 mM) was used to inhibit ricin toxicity. In view of the toxin concentration used (10^{-11} M) in our above described experiments, and since AZT is highly toxic for K562 cells at a concentration higher than 200 μM, we performed experiments using 40 and 200 μM AZT and RCA at 10^{-13} M concentration, greatly reducing the amount of toxin to enhance the effects of the drug on RCA-induced cytotoxicity. As it can be seen from Fig. 3A, at this concentration RCA inhibited protein synthesis by only 12%, but the presence of AZT abolished RCA cytotoxicity. In contrast, 24 h pre-treatment of cells with AZT increased RCA-induced cytotoxicity both at 40 and 200 μM AZT (23 and 31% inhibition of protein synthesis), but if fresh AZT was added again at the beginning of toxin incubation period, the presence of the drug, irrespective of its concentration, was always able to reduce these effects and inhibition dropped down to 13%, a value similar to that found in the absence of AZT.

On the other hand, the simultaneous addition of both chloroquine (10 μM) and AZT (40 or 200 μM) to K562 cells significantly enhanced RCA cytotoxicity, being protein synthesis inhibited by 33 and 47%, respectively (Fig. 3B). AZT pre-treatment again was able to rise RCA toxicity in the presence of chloroquine (55 and 43% inhibition of protein synthesis with 40 or 200 μM AZT), but the addition of fresh AZT counteracted this effect as shown before in the absence of chloroquine with inhibition reduced to 30 and 25% at 40 or 200 μM AZT, respectively.

As a further control, an experiment with 200 μM AZT to test the effect on TS toxicity was performed. The results shown in Fig. 3C demonstrate again that as for RCA, the new addition of AZT after pre-treatment can significantly decrease TS inhibitory activity on cellular protein synthesis.

Protein synthesis inhibition was also tested on HSB2 cells, a T-cell human leukemic line, to confirm that the effect of CQ and AZT on toxin activity was not linked to cell type. As shown in Fig. 4, under pre-AZT conditions the effect of the two drugs on the cellular toxicity of TS and RCA were similar to those observed with K562 cells, although the sensitivity of the HSB2 cells is different.

3.2. Confocal microscopy

We tested the effect of the exposure of cells to AZT on the cellular distribution of RCA and transferrin. As shown in Fig. 5A, the toxin is accumulated in a peri-nuclear structure which was previously characterized in other cell lines as the Golgi apparatus [31]. The presence of 40 μM AZT did not induce any change in this pattern, but exposure to 200 μM AZT was able to diffuse the fluorescence signal associated to the RCA molecule, and in some cells completely inhibited accumulation of the toxin in this subcellular compartment.

We observed an extensive accumulation of transferrin in K562 cells in a peri-nuclear region very similar to that

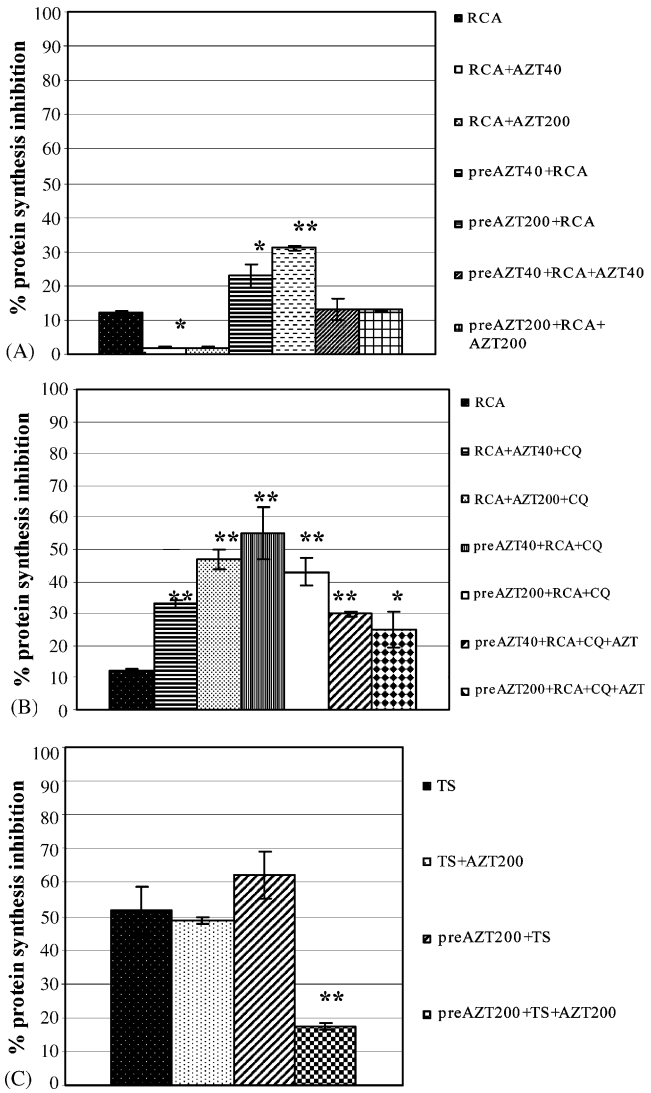

Fig. 3. Protein synthesis in K562 cells measured as incorporation of ^{3}H-leucine after incubation of cells with 10^{-8} M TS or 10^{-11} M RCA for 24 h as a function AZT concentration (40 or 200 μM) and chloroquine (10 μM) addition. Bars represent S.D. Symbols for single experimental conditions are reported near the plot. $^{**}p < 0.01$ or $^{*}p < 0.05$ vs. RCA (A), RCA (B), and TS (C).

populated by RCA, and furthermore we obtained a large co-localization of RCA and transferrin in the same conditions (data not shown). Opposite to that seen for RCA, AZT did not seem to produce changes in transferrin localization (Fig. 5B) even at the higher concentration (200 μM).

3.3. Effect of TS on cell proliferation and apoptotic cell death

The exposure of cells to TS-induced apoptotic cell death, was evaluated either as membrane permeability to EtBr and Acridine Orange and caspase-3 activation. TS can in fact increase the percentage of apoptotic cells by a factor 4 at 24 h and by a factor 8 at 48 h (Fig. 6A), while the caspase-3-like activity is increased by a factor 3 (Fig. 6B). The presence of AZT significantly enhanced the number of apoptotic cells and caspase activity at 24 h when cells have been previously exposed to the drug, or at 48 h when AZT has been added either at the same time or before TS.

Cell growth (Fig. 6C) was greatly inhibited by the presence of TS, and almost completely blocked by the addition of AZT together with the toxin.

4. Discussion

The potential use of targeted toxins for cancer treatment has been widely considered in the last two decades [3,5,6] and clinical trials have been carried out using immunotoxins, i.e. the chemical or recombinant conjugate of a toxin (or a toxin domain) with whole antibodies or their fragments. A systemic use of these new drugs has nevertheless been hampered by several side effects due to a-specific toxicity and immune responses. Great effort has been devoted to find new agents that could enhance the toxicity of chimeric toxins so that lower doses could be employed for therapy [32]. Because the tremendous toxic potential of some toxins (i.e. RCA, pseudomonas exotoxin, etc.) some investigations have instead looked for drugs able to inhibit toxin activity and being of pharmacological interest. In this perspective the weak base chloroquine, a well-known anti-malaric agent [33–36], is known to induce potentiation of some chimeric toxins [37,16], while AZT, the widely used anti-HIV drug [38], has been reported to inhibit RCA and pseudomonas exotoxin A activities [25] on several cell lines.

In this paper we investigated the effect of these two drugs (CQ and AZT) on the inhibition of protein synthesis induced by exposure of K562 cells to TS and RCA.

These cells have been previously investigated in our lab [39–41] and are characterized by a high level of expression of the Tf-receptor, thus they may be considered as a model for the study of Tf-mediated toxin internalization. Furthermore, K562 cells are reported to be resistant to drug-induced apoptotic death as a consequence of the expression of the p210BCR–ABL fusion protein [42–45], thus suggesting to study possible strategies directed to overcome this problem.

As demonstrated by the experiments reported in Fig. 1, TS is mostly unaffected by AZT at 40 μM concentration, whereas RCA is inhibited when AZT is added simultaneously to the toxin. We used AZT concentrations that are not far from those reached in the blood of AIDS patients under AZT therapy (0.5–10 μM). Previously reported data [26] on AZT inhibition of RCA toxicity demonstrated that the maximum effect could be reached when the drug is used at a concentration of 1 mM. Surprisingly, when we treated K562 cells with 40 μM AZT 24 h before RCA, its inhibitory effect on this toxin was reduced and could not be completely restored by renewal of the drug (i.e. by the further addition of 40 μM AZT at the moment of RCA

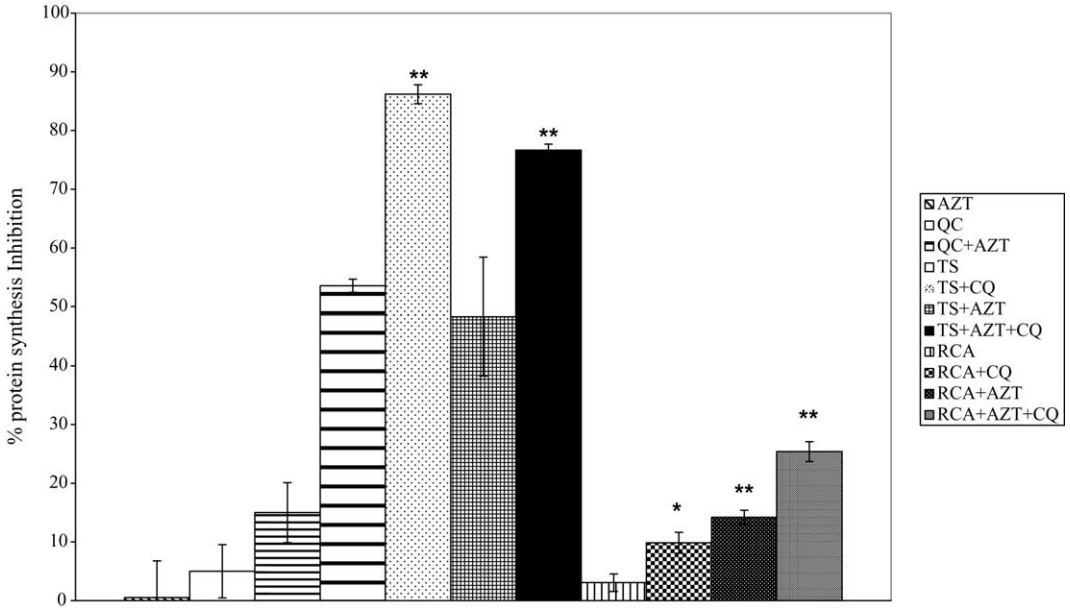

Fig. 4. Protein synthesis in HSB2 cells measured as incorporation of ^{3}H-leucine after incubation of cells with 10^{-8} M TS or 10^{-11} M RCA for 24 h after a pre-treatment with 40 μM AZT and in the absence or in the presence of 10 μM chloroquine. Data are reported as percentage of protein synthesis inhibition with respect to control untreated cells. Bars represent S.D. Symbols for single experimental conditions are reported near the plot. $^{**}p < 0.01$ or $^{*}p < 0.05$ vs. TS or RCA.

addition). Since the drug can be metabolically modified once inside the cell, it is possible that 40 μM AZT is not sufficient to exert its effect over 24 h, and that this effect may be reversible.

Even though TS seems to be unaffected by 40 μM AZT treatment, the presence of the drug can increase the apoptotic effect of TS on K562 cells, as shown in Fig. 5. Furthermore, the treatment of cells with chloroquine (10 μM) can increase TS toxicity if AZT is present. In these conditions, when 10^{-8} M TS was given to the cells for 24 h, the inhibition of protein synthesis was ~50%, but if CQ and AZT were added, the inhibition was increased to ~70%. It should be noted however that when AZT was used at 200 μM, TS toxicity was influenced, as the drug can decrease toxin efficiency when renewed after the pre-treatment, as for RCA (see below).

RCA toxicity at 10^{-11} M concentrations did not appear to be affected by these treatments under the same experimental conditions. Since RCA is highly toxic and we could have under-estimated the effect of drugs, we assayed the same drugs at lower RCA concentration (10^{-13} M). As it can be seen from the results of Fig. 3, RCA toxicity can be attenuated by the presence of 40 μM AZT, but in general pre-treatment of K562 cells with this drug induced an increase of RCA toxicity if the drug is not renewed in culture medium. By contrast, a reduction of the potentiating effect was observed if AZT was added again at the end of the pre-treatment period. AZT appears indeed to display two different effects: it can protect from RCA toxicity when it is added at the same time as RCA, but it can increase cellular sensitivity to the toxin if cells have been exposed to the drug 24 h before RCA. On the other hand, chloroquine added with AZT can induce a marked potentiating effect, and again if cells have been pre-incubated with AZT the increase of RCA toxicity in these conditions is significant (12% protein synthesis inhibition for RCA alone versus 55% inhibition with AZT pre-treatment followed by chloroquine exposure). However, even in this experimental protocol, if fresh AZT was added to the medium, we detected a reduction of toxicity (25–30% instead of 55%).

AZT pre-treatment of K562 cells was able to increase the pro-apoptotic potential of TS after 48 h incubation. These data on the whole demonstrate that CQ and AZT can be used to improve TS activity when added at pharmacological concentration, without affecting the inhibitory potential of the toxin itself. Since AZT is widely used as inhibitor of reverse transcriptase in HIV-infected patients [22], and CQ has been recently shown to have anti-HIV activity [46], their concomitant administration may be beneficial for AIDS patients. As far as transferrin-receptor concerns, it should be underlined that it has been targeted with chimeric toxins both in vitro and in vivo for a therapeutic approach against several cancer forms and particularly against gliomas [18,19]. In the light of these reports, TS can be potentially used to target proliferating cancer cells and the marked increase of its activity in the presence of CQ and AZT shown in our experiments may suggest the use of these drugs in HIV-infected patients suffering of cancer.

Furthermore, to verify that the effects observed on K562 cells were not limited to this cell line, we made preliminary experiments on HSB2 T-cell leukaemic line. As shown in Fig. 4, when tested in the same conditions as in Fig. 2, both

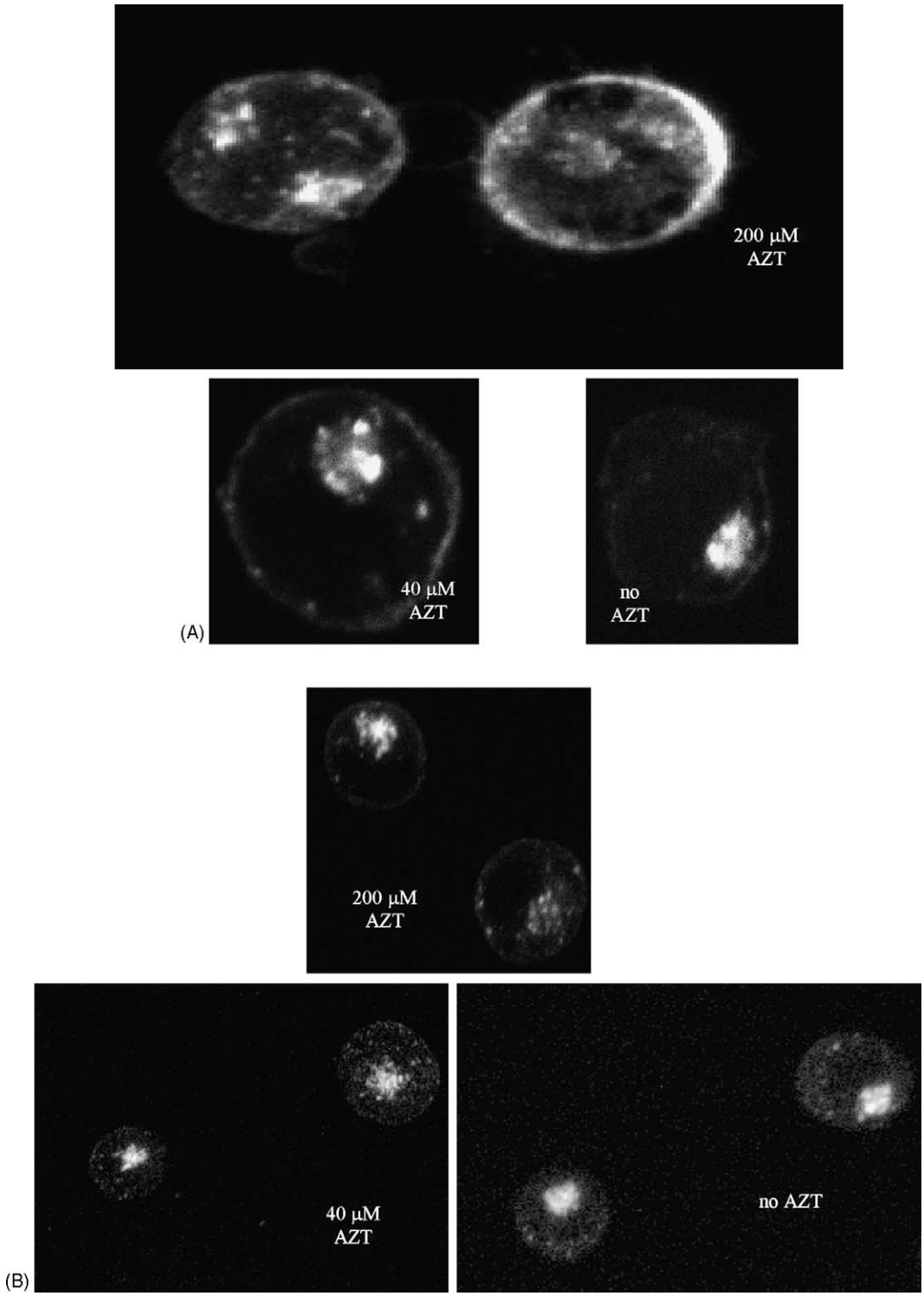

Fig. 5. Confocal microscopy of K562 cells incubated with (A) RCA (3×10^{-7} M) in three different conditions: +200 μM AZT, +40 μM AZT, no AZT and (B) transferrin (7.5×10^{-7} M) in the same conditions as in (A). The cells shown in the figure are taken from different microscopic fields. The two experiments have been carried out separately.

TS and RCA toxicities were increased by treatment with AZT and CQ. Being these cells of human tumor source, we can speculate (further experimental work is under progress with HSB2 antigen-specific immunotoxins) that the use of saporin or ricin immunotoxins is possible in patients under treatment with AZT and that the use of CQ may be beneficial in terms of increase of tumor cell death.

Besides the potential pharmacological use of CQ and AZT above reported, these drugs might be of help to better characterize the intracellular pathway of toxins. Our data

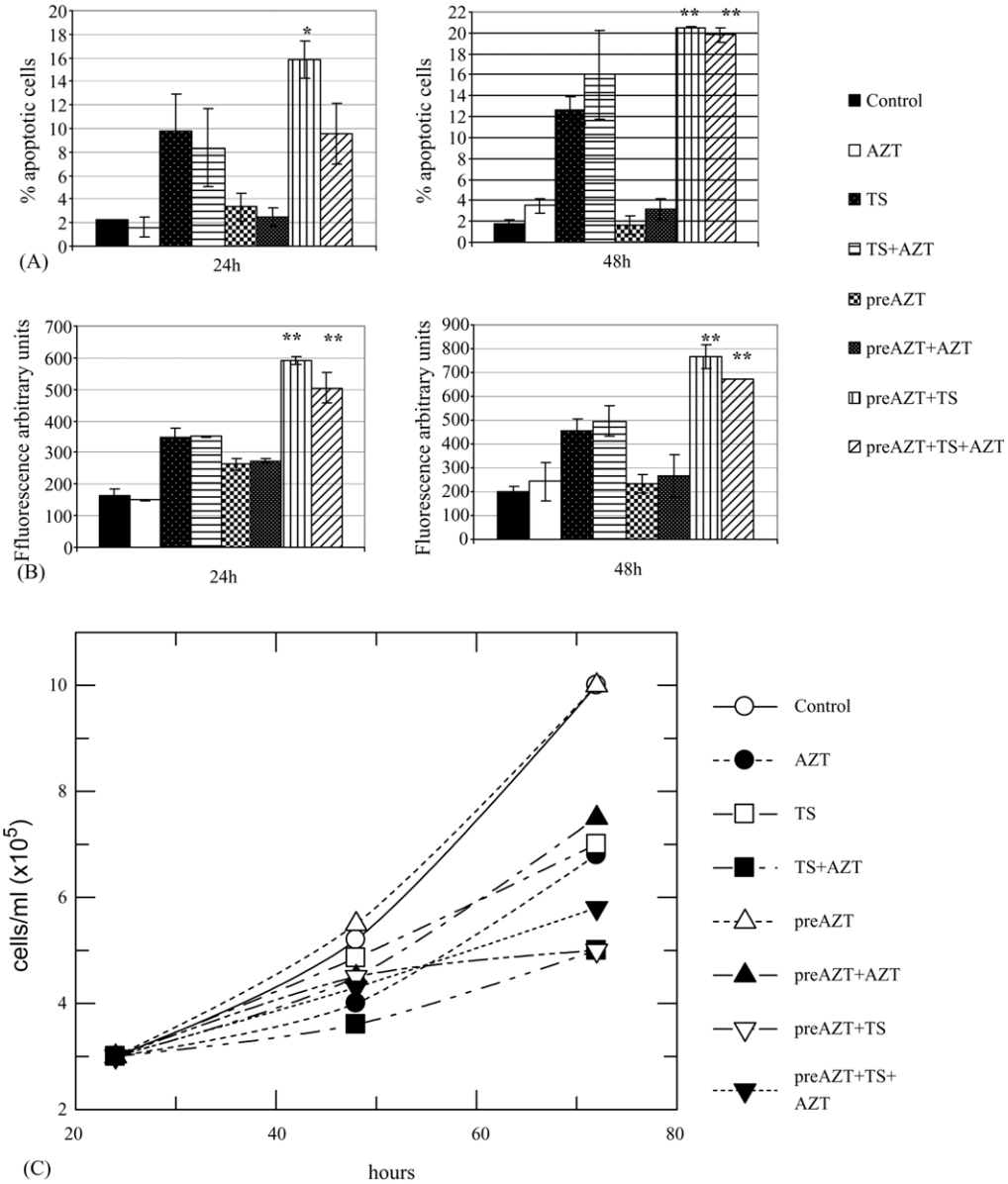

Fig. 6. Apoptotic effects of TS in the absence or in the presence of AZT (40 μM). Bars represent S.D. Symbols for single experimental conditions are reported near the plot. (A) Percentage apoptotic cells; (B) caspase-3-like activity; (C) cell growth; $^{**}p < 0.01$ or $^{*}p < 0.05$ vs. TS.

suggest that TS and RCA may have partially different intracellular delivery routes, as previously suggested [15]. It has been in fact demonstrated that AZT reduction of RCA toxicity is not caused by inhibition of its catalytic activity [26] and it has been suggested that the drug can interfere somewhere during the transport of the toxin inside the cell.

RCA endocytosis is a well-characterized process [11,47,48] involving transport of the toxin down the exocytic apparatus (i.e. the Golgi and the endoplasmic reticulum). Since RCA entry into cells is not influenced by the presence of AZT [25], it is plausible that the drug can interfere with the retrograde transport at the level of the Golgi or the ER.

TS endocytosis partially characterized in a previous paper [15] and our data concerning AZT effect in comparison with RCA, suggest that this chimera may partially differ from RCA in its pathway for delivery to the cell cytoplasm. To confirm this hypothesis, we have performed some experiments using fluorescent derivatives of transferrin and RCA to follow their endocytosis under the same experimental conditions used in the toxicity assays.

We observed that RCA is accumulated inside a perinuclear structure, possibly the trans-Golgi network (TGN). When cells were exposed to AZT, we could observe a diffusion of the toxin from these structures and in some cells an almost complete absence from the Golgi region, but only at 200 μM AZT. On the contrary, although transferrin was accumulated inside endocytic compartments partially overlapping those populated by RCA (i.e. the trans-Golgi), we did not see any effect on transferrin distribution inside these vesicles when cells were exposed to AZT.

By considering that the sensitivity in our experiments is not sufficient to observe few molecules moving from the TGN to the Golgi stacks and the endoplasmic reticulum (ER), we can only speculate on the fate of the bulk toxin and transferrin inside the cells. Anyway, from the data obtained in cytotoxicity experiments, we could suggest that the integrity of the Golgi may not be necessary for the transferrin-saporin chimera to kill the cells, while RCA toxicity appears much more dependent on a functional Golgi–ER system, as expected. Furthermore, chloroquine effects on both transferrin-saporin and RCA, suggest that this drug may influence their toxicities acting on a compartment that is independent from Golgi activities (i.e. endosomal system), possibly forcing the toxic moieties (saporin or ricin A-chain) to escape from endosomes directly to the cell cytoplasm and increasing the stability of these polypeptides inside acidic vesicles, due to chloroquine weak basicity. Further experiments are in progress to confirm this hypothesis.

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