Evidence that estramustine binds MAP-1A to inhibit type IV collagenase secretion

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Summary

Estramustine is a novel anti-microtubule drug shown to bind MAP-1 and MAP-2 (microtubule-associated proteins) in vitro. In this paper we have shown that estramustine specifically binds MAP-1A in Du 145a cells, resulting in disruption of MAP-1A microtubules and inhibition of type IV collagenase secretion. Immunofluorescence studies revealed that at 30 μM levels estramustine blocked type IV collagenase secretion by partial disruption of the MAP-1A microtubule networks. Immunoprecipitation studies with polyclonal antibodies provided quantitative evidence that 30–60 μM estramustine blocked secretion of a 105 × 10^6 M protein, type IV collagenase. Pulse-labeling experiments confirmed that the effect was not a result of inhibition of either protein synthesis or altered rates of type IV collagenase turnover. Finally, drug uptake studies with [3H]estramustine, scintillation counting and fluorography demonstrated that the principal target of the drug was MAP-1A. For the first time we have shown that the drug blocks secretion by binding MAP-1A and causing incomplete disruption of the microtubule networks.

Key words: estramustine, secretion, type IV collagenase.

Introduction

Estramustine is a novel anti-microtubule agent that consists of estradiol linked to nor-nitrogen mustard via a carbamate-ester linkage (Fex et al. 1984; Stearns et al. 1985). Attempts to identify the molecular targets of estramustine or estramustine phosphate indicate that it binds high molecular weight brain microtubule-associated proteins (MAPs 1 and 2) to prevent microtubule assembly or disrupt intact microtubules in vitro (Friden et al. 1987; Kanje et al. 1985; Stearns et al. 1985; Stearns and Tew, 1985; Stearns et al. 1988; Wallin et al. 1985) and in vivo (Stearns and Tew, 1985). The studies suggested that estramustine might be usefully employed to investigate microtubule- and MAP-dependent functions in cells. In this regard, one striking effect of estramustine is the inhibition of organelle motility. This inhibition is reversible and occurs at reduced dosages (<30 μM), well below that required for complete disruption of the microtubules (Stearns and Tew, 1985). Further studies are needed to determine if estramustine can bind one or more MAPs to block directly intracellular motility and/or secretion (or mitosis).

It is important to examine estramustine's effects on known specific secretory products, since not all membrane organelles are known to associate with the microtubules. Many of the published studies with anti-tubulin drugs (colchicine, vinblastine) have not determined if the drug blocks the transport and secretion of specific proteins. In addition, several reports have shown that anti-tubulin drugs will inhibit transport of some secretory and membrane proteins, whereas the transport of other proteins in the same cell were not affected (Arnheiter et al. 1984; Bennett et al. 1984; Blok et al. 1981; Rogalski and Singer, 1984; Rogalski et al. 1984).

Recently, we have studied the relationship between protease secretion and tumor cell invasion in metastatic cell lines. These studies of tumor cell invasion (Liotta et al. 1986; Wang and Stearns, 1988) showed that a 56 × 10^6 M, autocrine motility factor (AMF) (Liotta et al. 1986) (or conditioned medium from tumor cells containing the 56 × 10^6 M protein) stimulated both protease secretion and tumor cell invasion in vitro. We also showed that 30–60 μM estramustine inhibited the metastatic cell lines from migrating through a reconstituted basement membrane in response to the conditioned medium (CM) (Wang and Stearns, 1988). Mareel et al. (1988) confirmed our results and showed that low dosages of estramustine (less than 10 μM) prevented human prostate DU 145 cell invasion of embryonic chick heart fragments. The mechanism of drug action was not investigated, although the data suggested that inhibition was contingent on the drug's antimicrotubule effects (Mareel et al. 1988; Wang and Stearns, 1988), and possibly inhibition of protease secretion.

In this paper, we have utilized both immunofluorescence and quantitative methods to demonstrate that estramustine binds MAP-1A (not tubulin) and inhibits the secretion of type IV collagenase by cultured DU 145a cells.

Materials and methods

Cell culture

A subcloned cell line of DU 145 (Stone et al. 1978), termed DU 145as, was derived in this laboratory from a metastasis to the left leg. The cells were maintained and used below passage 10...
according to methods previously described (Wang and Stearns, 1988). Conditioned medium (CM) was prepared from A2058 melanoma cells by methods previously described (Wang and Stearns, 1988).

The DU 145a cells were allowed to attach in 160 mm² dishes for 2h prior to the addition of [3H]estradiol (1×10⁴ disintegrations/min per dish). Cells were labeled for 24h with 100 μCi of [3H]leucine per 150 mm² dish or 100 μCi per ml for 6h for immunoprecipitation studies (Knight et al. 1971). For protein and simultaneous nucleotide labeling cells were exposed to 100 μCi of [3H]thymidine and 100 μCi of [3H]leucine per 160 mm² dish (New England Nuclear, Boston) for 18h.

Purification and measurement of type IV collagenase levels

Collagenase was partially purified from the medium of DU 145a cells utilizing previously published techniques (Barrett, 1973; Liotta et al., 1979; Liotta et al., 1981; McCroskey et al., 1975; Stone et al., 1978; Turpeenniemi-Hujanen et al., 1986).

The cellular collagenase was purified by the following methods. The medium was collected, the cells washed twice with phosphate-buffered saline (PBS), harvested by scraping with a rubber policeman, and homogenized in 2 ml PBS at 4°C with 20 up-and-down strokes of a teflon to glass Wheaton homogenizer (5 ml volume). The whole cell extract was centrifuged at 180,000 g for 1h at 4°C in a Beckman TL100 centrifuge. The collagenase was precipitated from a high-speed supernatant with 40% ammonium sulfate, and dialyzed against PBS for 4h. A 10⁵x10⁶Mr type IV collagenase was purified from the above preparations by absorption to antibody bound to a protein A filter (New Brunswick Instruments, Trenton, NJ). Collagenase was eluted from the column with 0.3 M KCl and the eluates (usually about 1.0 ml) were dialyzed against collagenase reaction buffer, aliquoted and frozen at -80°C. Affinity-purified polyclonal antibodies raised against a 105x10⁶M, type IV collagenase from DU 145 cells (Wang and Stearns, 1988) were used in these studies.

MAP-1A purification

DU 145a cells were grown to log phase at about 80% confluence and exposed to [3H]estradiol at 20 ng ml⁻¹ for 3h before harvesting. The drug was prepared and used by methods previously described (Wang and Stearns, 1988). The cells were washed and harvested (see above), then washed twice with a microtubule stabilizing buffer (MSB) containing 0.1 M Pipes, 2 mM EGTA, 2 mM MgCl₂, pH 7.2, at 4°C. Cells were homogenized in MSB at 1:1 (w:v) at 4°C to stabilize the microtubules and the homogenate was centrifuged at 20,000 g for 30 min (in a SS34 Beckman Rotor) to pellet cell debris. The supernatant (≈1 ml) containing soluble microtubule proteins was immediately eluted on an A5m agarose (Bio-Rad, Inc., Richmond, CA) sizing column (1.5 cm × 36 cm) pre-equilibrated with PBS at 4°C.

Fractions 11–12 were pooled (≈1 ml) and 6S tubulin was purified from pig brain (Murphy et al. 1977) added at 2 mg ml⁻¹ final concentration. The fractions were dialyzed against MSB (1 litre), 2 mM GTP added and incubated for 30 min at 37°C to assemble the microtubules. The sample was centrifuged through a 15% sucrose cushion at 150,000 g for 1h, and the microtubule pellet collected. Without adding more exogenous brain tubulin the microtubules were cycled twice by the assembly–disassembly protocols of Murphy et al. (1977) and the final microtubule pellet was resuspended in MSB containing 10 μM taxol at 37°C. The taxol-stabilized microtubules were extracted with 0.3 M KCl for 10 min at 37°C and the sample was centrifuged through a 15% sucrose cushion to separate the microtubules from the MAP-enriched supernatant. Samples were taken for SDS-PAGE (Laemmli, 1970), protein measurements (Lowry et al. 1951), Western blotting (Towbin et al. 1970), scintillation counting and fluorography. The gels were silver stained according to the method of Morrissey (1981).

Fig. 1. Double-labeled immunofluorescence images of untreated DU 145a cell stained with: (A) tubulin polyclonal antibody; and (B) a MAP-1A monoclonal antibody. (C) A spindle labeled with MAP-1A antibodies. x1200.
Immunofluorescence studies

Immunofluorescence studies were carried out according to methods described previously (Stearns and Binder, 1987). Double labeling was carried out with polyclonal tubulin antibodies (Wang et al., 1987), monoclonal MAP-1A and MAP-1B antibodies (Bloom et al., 1984; Bloom et al., 1985), courtesy of G. Bloom, University of Texas, Dallas; monoclonal 210 x 10^3 Mr antibodies (Bulinski and Borisy, 1980) courtesy of J. C. Bulinski, N.Y.U., New York; and monoclonal tau antibodies (Binder et al., 1985) courtesy of L. Binder, University of Alabama, Birmingham. Dilutions of 1:200 were used for the primary antibodies and 1:500 or 1:400 for the secondary antibodies.

Results

Immunofluorescence studies

Immunofluorescence was utilized to monitor the expression of MAP-1A and a 105 x 10^3 Mr type IV collagenase in the cultured DU 145 cells. In untreated cells or cells exposed to estramustine's constituents, 30 μM estradiol and non-nitrogen mustard, the microtubules and the associated MAP-1A formed normal centrosomal arrays in DU 145 cells (Stearns et al., 1988) and DU 145a cells (Fig. 1). Fig. 2 further shows that the cells contained some type IV collagenase localized in vesicles that were scattered throughout the cells.

After a 3 h exposure to 30 μM estramustine the cell shape remained the same (Fig. 3A) but the microtubule population was reduced severely. MAP-1A remained associated with the microtubules (Fig. 4A,B) but the tubules were often broken into pieces and no longer concentrically arrayed from the centrosomal complex (Figs 2A, 3B, 4A,B). The intensity and extent of type IV collagenase labeling was increased significantly (i.e. more vesicles) over that observed in untreated cells (compare Figs 2B and 3C). At higher drug dosages (60 μM for 1 h) the MAP-1A microtubules were almost completely removed and the type IV collagenase antibody staining was greatly enhanced (data not shown).

Following removal of 30 μM estramustine the cells slowly re-formed their centrosomal microtubules after 1–2 h and the intensity of type IV collagenase antibody labeling was diminished to control levels observed in untreated cells. There was very poor recovery following exposure to 60 μM drug for 3 h, however.

Radiolabeling studies with [3H]estramustine

Quantitative radiolabeling studies confirmed that the drug was rapidly incorporated by DU 145a cells over a 0–6 h period (Fig. 5). The amount of drug uptake was increased as a function of increasing the cell density from 1 x 10^6 cells ml^-1 to 2 x 10^6 cells ml^-1. Most of the drug incorporated (about 70% of the total available) was taken up within 2–3 h at 37°C, and at about 3 h the amount of drug uptake reached a plateau. In comparison, drug uptake was limited at 4°C even after 6 h incubations (Fig. 5).

To investigate the possible molecular targets of estramustine the cells (≈4 x 10^6) were incubated with [3H]estramustine (20 ng ml^-1 for 3 h). The cells were homogenized to obtain a crude cell extract, the preparation centrifuged, and the high-speed supernatant chromatographed using an A5m column. SDS–PAGE showed that column fractions 10 and 11 contained tubulin and microtubule-associated proteins (data not shown). SDS–PAGE further showed that the microtubules were removed from fractions 10 and 11 (Fig. 6, lane 1), and partially purified by temperature cycling (twice) methods. These microtubules were enriched for the 330 x 10^3 Mr, MAP-1A (Fig. 6, lane 2). The microtubules were stabilized with taxol and the 330 x 10^3 Mr protein was removed in the supernatant following salt extraction and centrifugation (Fig. 6, lane 4). Western blots confirmed that the 330 x 10^3 Mr protein was MAP-1A (Fig. 6, lane 5). Western blots with MAP-1B, the 210 x 10^3 Mr MAP and tau antibodies indicated these antigens were not present in any of these fractions (data not shown).

Scintillation counting showed that most (95%) of the [3H]estramustine was associated with the high-speed supernatant of the crude cell extracts. About 88% of the total counts (i.e. 22 x 10^-8 disintegr min^-1) were retained in...
the A5m column fractions 10–11 (Fig. 7 and Table 1). The microtubules removed from A5m column fractions 10–11 contained about 82% (18 × 10⁻⁸ disints min⁻¹) of the total counts. Little or no label (<3 × 10⁻⁴ disints min⁻¹) was found in the microtubule-depleted supernatant (super- natant - I) of fractions 10–11. Finally, the MAP-IA-rich supernatant obtained from salt extraction of the twice- cycled microtubules contained about 83% (15 × 10⁻⁸ dis- ints min⁻¹) of the [³H]estramustine. Note that the labeled drug did not co-precipitate with the salt-treated taxol microtubules and associated intermediate filaments or microfilaments.

In absolute terms, the [³H]estramustine associated with the purified MAP-IA supernatant represented about 60% of the total counts found in the initial crude cell extract. On the basis of these data (Table 1), we calculated that the amount of [³H]estramustine mg⁻¹ protein was increased by about 120 000-fold during purification of MAP-IA from the crude cell extracts. The drug must be tightly bound to the MAP-IA molecule, since the samples were routinely dialyzed overnight at 4°C prior to counting and 0.3 M KCl failed to separate [³H]estramustine from MAP-IA.

It is possible that a minor component representing less than 1% of the protein or a carbohydrate or lipid moiety might be responsible for estramustine binding. The latter possibility was ruled out in experiments where 0.1% lipase, or 1% endoglycosidase H or 0.1% neuraminidase (freshly prepared) were added to the purified MAP-IA preparations for 10 min at 37°C followed by dialysis overnight at 4°C. Scintillation counting revealed that the enzymes did not diminish the amount of [³H]estramustine bound per mg of protein in the MAP-IA fractions (data not shown).

Fluorography of the above MAP-IA samples confirmed that [³H]estramustine was associated with the MAP-IA molecule at each step in the purification (Fig. 8). Note that the amount of purified MAP-IA loaded in lane 4 was higher than that in lanes 1–3 and the resulting [³H]estramustine signal was also more intense. Fluorography also confirmed that lipase, endo H and neuraminidase did not remove or diminish the amount of [³H]estramustine binding to the MAP-IA protein (data not shown). All enzymes were tested at 37°C under optimal conditions for 30 min.

Effects of estramustine on type IV collagenase secretion

Quantitative immunoprecipitation measurements of the total amounts of radiolabeled type IV collagenase secreted showed that cells exposed to 30–60 μM estramustine
secreted little or no collagenase over the entire 5 hr period tested (Table 2). The drug also inhibited protein secretion in general during the 0–5 h incubation time (Table 2).

Table 2 shows that in the absence of drug, the total amount of protein secreted (8×10⁻⁴ dis/units min⁻¹) is twice that of the total type IV collagenase secreted (4×10⁻⁴ dis/units min⁻¹). When the cells were exposed to 30 μM estramustine, the extent of total protein and type IV secretion dropped to 1.5×10⁻³ and 1.1×10⁻³ dis/units.

Fig. 5. [³H]estramustine uptake by DU 145α cells plated at (●, ○) 2×10⁵ cells ml⁻¹; (●, □) 1×10⁶ cells ml⁻¹, uptake was measured at 37°C (●, □) and 4°C (○) for 0–6 h periods. Note that 4×10⁻³ dis/units min⁻¹ were originally added to each dish and 70% of the total counts were taken up by the cells after 6 h. Values were averaged from 3 dishes (experiments).

Fig. 6. A silver-stained SDS-PAGE (7% gel) of: lane 1, MAP- and microtubule-depleted A549 column fractions 11, 12; lane 2, twice-cycled microtubules; lane 3, salt-extracted taxol microtubules; lane 4, MAPs removed from twice-cycled microtubules; and Western blots with MAP-1A antibody of: lane 5, twice-cycled microtubules; and lane 6, the MAPs in lane 4. The lines (left side) indicate molecular weight markers of 200, 116, 92, 66 and 45 (×10⁻³).

4A

4B

Fig. 4. Part of a DU 145α cell exposed to 30 μM drug for 3 h and double-labeled with: (A) tubulin polyclonal; and (B) MAP-1A monoclonal antibodies. ×2500.
min⁻¹, respectively. Likewise, with 60 μM drug these levels were further diminished to background levels. A known anti-tubulin drug, nocodazole, had an identical effect on both protein and collagenase secretion. However, unlike estramustine, nocodazole produced cell rounding and some displacement of the Golgi apparatus.

Control experiments showed that in cells exposed to 30 μM estradiol and nor-nitrogen mustard the total amount of labeled protein and collagenase secreted was similar to that for untreated cells. [³H]thymidine labeling, and Trypan Blue dye-exclusion tests demonstrated that there was limited cell death or leakage of material in response to 30–60 μM estramustine.

Immunoprecipitation was further employed to determine if estramustine has a time-dependent effect on protein synthesis and cytosolic type IV collagenase levels.

Fig. 8. Fluorography of MAPs fractions isolated from [³H]estramustine (20 ng for 3 h at 37°C)-treated cells, showing: lane 1, crude whole cell extract; lane 2, A5m column fractions 11–12; lane 3, microtubules, twice cycled; lanes 4, 5, purified MAPs. A single band at 330×10⁻³Mr was labeled with [³H]estramustine. No labeling was observed if the purified MAPs were electrophoresed in the presence of 0.1% β-mercaptoethanol (lane 5). An absence of labeling was observed in the MAP-depleted supernatants of crude cell extracts (lane 6) and A5m column fractions 11–12 (lane 7) or in other A5m column fractions (i.e. fraction 45, lane 8).

In these experiments, the DU 145g cells were plated at 4×10⁻⁷ ml⁻¹ (in 20 ml) for 3 h, then labeled for 4 h with 100 μCi ml⁻¹ [³H]leucine while being exposed to CM (20%) and drug for a 0–5 h period (Fig. 9). The data showed that in the absence of drug, or in the presence of 30 μM estradiol and nor-nitrogen mustard, the cytosolic levels of labeled [³H]collagenase increased over the first 3 h and then remained relatively constant for the remainder of the period tested. In comparison, in the presence of 30 μM and 60 μM estramustine, the cellular levels of labeled enzyme increased linearly from 0 to 5 h.

The corresponding immunoprecipitation measurements of the total type IV collagenase released in the medium showed that in the absence of drug, the levels increased during the initial 0–3 h period, reaching a constant level at 3 h. In the presence of 30 μM estradiol and 30 μM nor-nitrogen mustard, a similar increase and plateauing effect was observed. In comparison, when estramustine was present at 30 μM and 60 μM levels secretion was totally inhibited and little or no [³H]collagenase was detected in the medium (Fig. 9). Immunoprecipitation studies further showed that after 2 h recovery in fresh medium (from 30 μM drug for 3 h) the counts in the whole cell extracts dropped to about 1×10⁻⁴ disintegrations min⁻¹, while the counts in the medium increased to about 0.8×10⁻⁴ disintegrations min⁻¹, indicating that collagenase was released from the cells.

<table>
<thead>
<tr>
<th>Drug (μM)</th>
<th>Total protein</th>
<th>Type IV collagenase</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8.0×10⁻⁴</td>
<td>4.0×10⁻⁴</td>
</tr>
<tr>
<td>30</td>
<td>1.5×10⁻³</td>
<td>1.1×10⁻²</td>
</tr>
<tr>
<td>60</td>
<td>5.0×10⁻²</td>
<td>0.5×10⁻²</td>
</tr>
<tr>
<td>30*</td>
<td>7.2×10⁻⁴</td>
<td>3.8×10⁻⁴</td>
</tr>
<tr>
<td>1†</td>
<td>1.2×10⁻²</td>
<td>1.0×10⁻²</td>
</tr>
</tbody>
</table>

The cells were pulse-labeled for 5 h with 100 μCi of [¹⁴C]leucine ml⁻¹ and incubated in fresh serum-free medium containing 20% conditioned medium. Scintillation counting was used to measure: (1) total protein levels in the medium and (2) type IV collagenase immunoprecipitated from the medium. Pulse-labeling studies with [³H]thymidine showed that cell death was not increased above normal by drug (1.1×10⁻² to 3×10⁻⁴ disintegrations min⁻¹ in medium). The data represent values averaged from measurements of the medium from three separate 150 mm² dishes with cells plated at about 60% confluence.

* 30 μM estradiol plus 30 μM nor-nitrogen mustard.
† 1 μM nocodazole.

Table 2. Effects of both total protein and type IV collagenase secretion

Fig. 7. A5m column fractions (0.5 ml/fraction) of the crude high-speed supernatant of DU 146g cells. Protein (X—X), absorbance at 550 nm. [³H]estramustine (O—O).
following removal of the drug. Little or no secretion was detected following removal of 60 μM estramustine.

Control experiments showed that in the presence of the protein synthesis inhibitor, 20 μM chloramphenicol, the levels of cytosolic [3H]leucine-labeled type IV collagenase were almost undetectable at 2 × 10⁻⁷ disintegrations/min and the total collagenase secreted remained near zero or background levels of 1 × 10⁻⁷ disintegrations/min for 0–5 h cultures.

**Discussion**

We have obtained quantitative evidence that estramustine is rapidly taken up by DU 145a cells and that it inhibits secretion of a critical protease essential for tumor cell invasion in vitro (Liotta et al. 1986). For these studies, we have utilized a prostatic tumor cell line subcloned for enhanced invasive and metastatic capabilities (Wang and Stearns, 1988). When cultured on a type IV collagen substratum the DU 145a subclone secreted excessive amounts of type IV collagenase in response to CM of A2058 melanoma cells that contained a specific 56 × 10³ Mr autocrine motility factor (Liotta et al. 1986; Wang and Stearns, 1988). Thus, it was feasible to measure estramustine’s dosage-dependent effects on the expression and secretion of type IV collagenase following activation by a specific signal in the CM.

The drug also blocked protein secretion in general but it appeared to exert minimal non-specific toxic side-effects (such as disruption of the Golgi apparatus, which occurred when nocodazole was applied) as a result of its antimicrotubule effects. It remains to be shown if estramustine blocks the secretion of all proteins at the same step in processing or by a similar mechanism of drug action.

The results have demonstrated that a causal relationship exists between estramustine binding to MAP-1A, a partial disassembly of microtubules and complete blockage of type IV collagenase secretion in activated DU 145a cells. The data correlate closely with earlier light-microscopic observations showing that 30 μM estramustine rapidly blocks intracellular vesicle transport (Stearns and Tew, 1986). We postulate that inhibition of secretion might arise directly from drug binding to MAP-1A or a MAP-1A complex containing tubulin and the vesicle transport motor (i.e. kinesin), or directly from the partial disassembly of the MAP-1A microtubules. The latter possibility is suggested by immunofluorescence data that showed that the MAP-1A microtubules were disrupted following drug treatment. The microtubules that remained were disoriented, sometimes broken into pieces, and no longer arranged in the concentric centrosomal arrays that are observed in intact cells.

Estramustine’s association with MAP-1A might interfere with normal microtubule assembly–disassembly events (Walker et al. 1988) and thereby indirectly prevent collagenase transport to the cell surface. Studies with
higher drug levels (60 μM) confirm these observations and show that the drug induces a complete and irreversible destruction of the microtubules, which appears to block secretion. Still, we cannot rule out the possibility that MAP-1A may also associate with other cytoskeletal elements or organelles (Asai et al. 1985; Sato et al. 1983) such as the plasma membrane (e.g., perhaps in a dephosphorylated state), and have other unknown functions (Arneil et al. 1984; Fex et al. 1984; Wiche et al. 1984; Wiche et al. 1986) that estramustine also blocks. Alternatively, estramustine may bind other proteins (Fex et al. 1984) or other MAPs (Friden et al. 1987; Stearns and Tew, 1988). For example, Friden et al. (1987) have reported that estramustine binds MAP-2 and tau in brain extracts. We found no evidence of the drug binding other proteins or other known MAPs in the drug-uptake studies. We utilized antibodies raised against three well-characterized MAPs (Binder et al. 1985; Bloom et al. 1985; Bulinski and Borisy, 1986) to determine if these proteins were present. Immunofluorescence labeling and Western blotting with MAP-1B monoclonal antibodies (Bloom et al. 1985), a 210×10^6 Mₗ HeLa MAP (Bulinski and Borisy, 1980) and tau antibodies (Binder et al. 1985) indicated that these molecules were not expressed in DU 145 cells. Only MAP-1A was found in DU 145c cells. Note that, although we did not identify any of the faint high molecular weight bands in the partially isolated microtubule preparations, these proteins co-purified with the salt-stripped microtubules, which did not bind [3H]estramustine.

Despite the fact that our data strongly implicated MAP-1A and microtubules as the principal targets of estramustine, it is still possible that alternative drug-sensitive transport pathways also exist. Finally, since the microtubules are partially resistant to relatively low dosages of estramustine (i.e. 30 μM), it is possible that the drug's weak affinities for other proteins affect processes stimulated by the CM. For example, the drug may block receptor binding by the 86×10^6 Mₗ AMP in CM and prevent secretory vesicle fusion with the plasma membrane.

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References


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