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Drug-Induced Glycosaminoglycan Storage: Dose-Dependent Changes in the Pattern of Accumulated Glycosaminoglycans in Cultured Bovine and Human Fibroblasts

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ABSTRACT. The present study determines the amounts and patterns of glycosaminoglycans stored in cultured corneal fibroblasts after treatment with tilorone and three related compounds. The compounds have immunomodulatory properties and have been shown to impair the lysosomal degradation of glycosaminoglycans as a side effect. This side effect has been described as drug-induced mucopolysaccharidosis because the induced lysosomal storage of glycosaminoglycans leads to cellular lesions resembling those in patients with inherited mucopolysaccharidosis. In the present study, the dose-dependency of glycosaminoglycan storage was analyzed after treatment (96 hr) of bovine corneal fibroblasts. The investigated drug concentrations ranged from low concentrations inducing cytological lesions typical of drug-induced mucopolysaccharidosis to high concentrations at the borderline of cytotoxicity. The intracellular amounts of dermatan sulfate, heparan sulfate, and chondroitin sulfate were quantified by densitometric scanning of Alcian Blue-stained bands after electrophoresis. All investigated compounds induced a predominant dermatan sulfate storage (3–4-fold accumulation) at low drug concentrations. With rising drug concentrations, a shift of the pattern of stored glycosaminoglycans was observed, characterized by the additional accumulation of heparan sulfate (up to 5-fold of control levels). In cultured human fibroblasts, tilorone also caused a marked dermatan sulfate storage, reaching maximum values at 5 μM and marked heparan sulfate storage at 20 μM. The present data provide evidence: (a) that selective dermatan sulfate accumulation is a characteristic feature of drug-induced glycosaminoglycan storage in cultured bovine and human fibroblasts, if these cells are treated with low concentrations (≤ 5 μM), that are likely to reflect the situation *in vivo*; and (b) that additional heparan sulfate storage is induced *in vitro* only by treatment with high concentrations that induce nonspecific cellular lesions. BIOCHEM PHARMACOL 52;9:1331–1337 , 1996. *Copyright © 1996 Elsevier Science Inc.*

KEY WORDS. tilorone; dicationic amphiphilic drugs; glycosaminoglycans; dermatan sulfate; lysosomal storage; lysosomotropic agents

The synthesis of GAG† chains takes place in the Golgi apparatus, where the carbohydrate residues become attached to protein cores. The resulting PGs are subsequently secreted into the extracellular space or are integrated into the cell membrane-associated (pericellular) PG pool [1]. Degradation of intracellular and pericellular PGs occurs in the endosomal and lysosomal compartments after endocytosis. The present study deals with drug-induced lysosomal GAG storage that is, as a side effect, induced by a group of dicationic compounds. These compounds have been considered for medical use because of their immunomodulatory properties [2–4]. Investigations concerning this side effect in a rat model [5–7], and cultured cells of several species [8–11], revealed that these compounds interfere with the lysosomal catabolism of GAGs.

It has been shown that *in vitro* at least two mechanisms play a role in the induction of this storage phenomenon. First, at low concentrations of tilorone, complex formation between GAGs and tilorone molecules within the lysosomes has been suggested to impair GAG degradation [6, 7]. This hypothesis was indirectly supported by the finding that a selective storage of free DS chains in cultured fibroblasts [12] is paralleled by a specific interaction between tilorone and free DS chains in aqueous solutions, as shown by NMR spectroscopy [13]. Second, at high drug concentrations *in vitro*, the above mechanism becomes superimposed by a nonspecific effect [14, 15] that tilorone shares with other weak bases, such as ammonia. Those weak bases induce mistargeting of lysosomal proenzymes because of elevated endosomal pH values. Tilorone concentrations ≥ 10 μM enhance the secretion of lysosomal proenzymes in cultured bovine fibroblasts [15] and induce the occurrence

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† *Abbreviations:* GAG, sulfated glycosaminoglycans; DS, dermatan sulfate; HS, heparan sulfate; CS, chondroitin sulfate; PG, proteoglycan.

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of nonspecific morphological lesions. Biochemical analysis of stored material revealed that, after treatment with 20 μM tilorone, 50% of accumulated GAGs were stored as integral parts of PGs rather than as free chains, and that additional storage of HS occurred [12]. It is quite unlikely that the latter mechanism is responsible for GAG storage *in vivo*, because (a) drug concentrations > 10 μM will not be reached in the extracellular fluids of intact organisms, and (b) the morphological lesions occurring in lysosomes of intact organisms could be reproduced in cultured cells only by low drug concentrations.

The aim of the present study was to characterize the concentration-dependency of tilorone-induced storage of DS, HS, and CS. Furthermore, the effect of three other substances with molecular structures similar to tilorone was analyzed. The three congeners of tilorone (Fig. 1) have previously been shown to be as potent as tilorone in causing GAG storage in rats and cultured cells [5, 6, 10, 11]. The investigation of these compounds invites considerations on structure-activity relationships, because they are characterized by different aromatic ring systems: fluorenone (tilorone), fluorene (RMI-10.233), acridine (CL-90.100), and anthraquinone (RMI-10.024).

The present data suggest that all investigated compounds are capable of predominantly interfering with DS degradation at low concentrations. Additionally, it was found that low concentrations of tilorone predominantly induce storage of DS in human peridontal fibroblasts, as well.

MATERIALS AND METHODS

Materials

Tilorone (2,7-bis[2-(diethylamino)ethoxy]fluorene-9-one) was kindly provided by Marion Merrell Dow Research Institute, Marion Merrell Dow, Inc. (Cincinnati, OH, U.S.A.) and CL-90.100 (3,6-bis[2-(diethylamino)ethoxy]acridine) by the American Cyanamid Co. Lederle Laboratories (Pearl River, NY, U.S.A.). RMI-10.024 (2,6-bis[2-(diethylamino)ethoxy]anthraquinone) and RMI-10.233 (2,7-bis[4-(piperidino)butyryl]fluorene) were from Sigma (München, Germany). The following materials were purchased from the suppliers indicated: media and solutions for cell culture from Biochrom (Berlin, Germany) and Boehringer (Mannheim, Germany); plastic culture flasks from Falcon (Becton-Dickinson, Heidelberg, Germany); DEAE-Trisacryl M IBF from Serva (Heidelberg); cellulose acetate membranes from Bender & Hobein (Moers, Germany); ammonium chloride, dermatan sulfate (bovine mucosa), chondroitin-4 sulfate (bovine trachea), heparan sulfate (bovine kidney), chondroitin ABC lyase (*Proteus vulgaris*, EC 4.2.2.4), chondroitin AC lyase II (*Arthrobacter aurescens*,

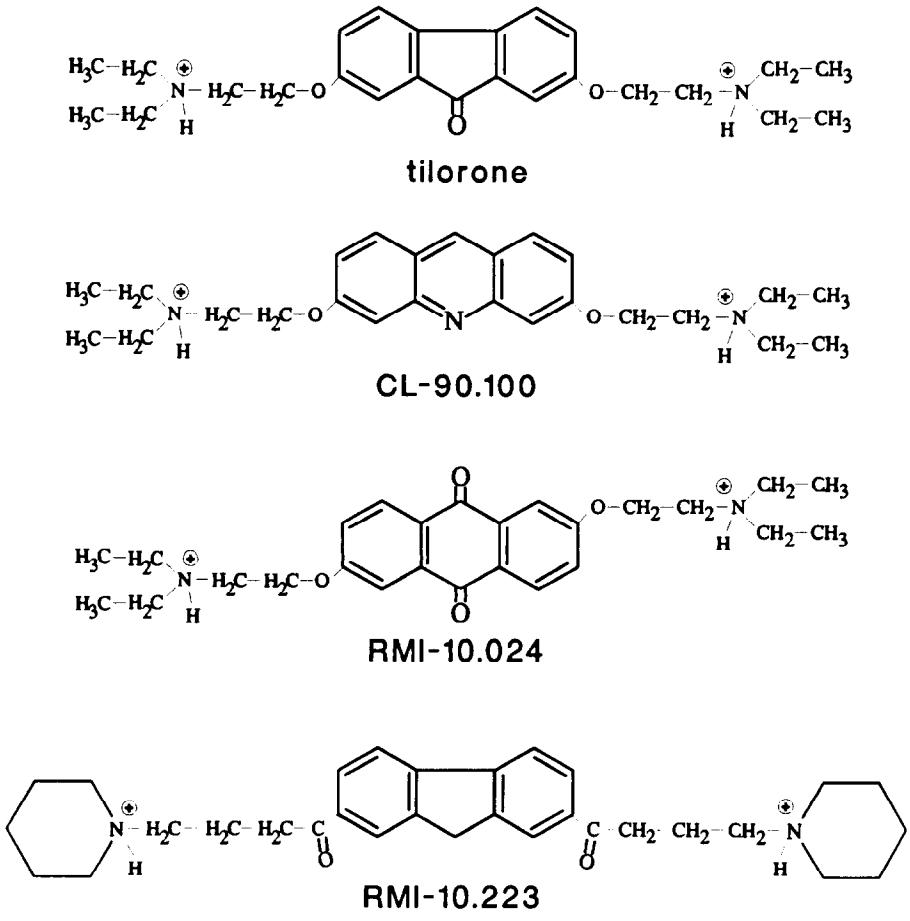

FIG. 1. Molecular structures of tilorone, CL-90.100, RMI-10.024, and RMI-10.233 in the protonized state.

EC 4.2.2.5) and heparin lyase III (*Flavobacterium heparinum*, EC 4.2.2.8) from Sigma (Munich, Germany) and Alcian Blue 8 GS from Fluka (Buchs, Switzerland). The other chemicals were of analytical grade and obtained from Merck (Darmstadt, Germany) and Sigma (Munich, Germany).

Cell Culture

The bovine corneal fibroblasts were obtained by the technique described previously [9] and human desmodontal fibroblasts were derived from desmodontal tissue explants of a healthy male [9]. The cells were grown in Eagle's minimal essential medium (MEM) with Earle's salts supplemented with 10% (v/v) fetal calf serum, nonessential amino acids, and penicillin/streptomycin (100 IU/100 μg per mL) at 37° and 5% CO_2. The split ratio for confluent monolayers for bovine and human fibroblasts was 1:3 and 1:2, respectively; cells from passages 4–8 were used.

Drug Treatment

The fibroblasts were seeded into 75 cm^2 plastic flasks. When monolayers had reached confluency, the experiments were started by supplying fresh medium containing the drugs. Exposure lasted 96 hr with no change of medium during this period. The ratio of medium volume per culture surface was kept constant at 0.37 mL/cm^2 in all experiments. Cultures kept simultaneously without addition of drugs served as controls.

GAG Analysis

GAGs were isolated from three compartments. Secreted (extracellular) GAGs were obtained from the culture medium. After removal of the medium, cells were washed with PBS and harvested by means of trypsin (0.1% (w/v), 10 min, 37°C). Cells were centrifuged and pericellular GAGs obtained from the supernatant. The fibroblasts were homogenized prior to isolation of intracellular GAGs.

Subsequently, GAGs from the three compartments were isolated as described previously [13]. Briefly, after a β-elimination reaction, GAGs were isolated by means of anion-exchange chromatography (DEAE-Trisacryl M IBF). After dialysis, samples were lyophilized and dissolved in appropriate volumes of deionized water. The separation of GAGs with respect to the different carbohydrate backbones was achieved by cellulose acetate electrophoresis in 0.1 M $Ba(CH_3COO)_2$-buffer [16]. In addition, GAGs were identified by enzymatic degradation procedures. Parallel digestions were performed with chondroitin ABC lyase, chondroitin AC II lyase, and heparin lyase III. Digestion with each of the enzymes was performed with 0.05 units of the respective enzymes in a total volume of 200 μL. The samples were incubated (37°C) for 30 min in the case of chondroitin ABC lyase and chondroitin AC II lyase, and for 60 min in the case of heparin lyase III. After the depolymerization procedures, the samples were applied to electrophoresis. Because the β-elimination reaction resulted in a cleavage of the protein-carbohydrate linkage region, it should be emphasized that the data did not allow conclusions as to whether the indicated GAGs originated from intact proteoglycans or had existed as free GAG chains in the culture system. This premise underlies the way the abbreviation GAG is used throughout the text.

The quantitative analysis of the GAG pattern was accomplished by densitometric scanning [568 nm] of Alcian Blue-stained strips. Samples were quantified by means of standard curves established by plotting known amounts of reference GAGs vs the area under the corresponding densitometric curves. After treatment with the indicated concentrations of drugs, the amount of intracellular GAGs was expressed as the percentage of untreated control cells, cultured simultaneously under identical conditions.

RESULTS

Bovine Corneal Fibroblasts

In a preceding study, DS was shown to account for 70% of total GAGs present in the intracellular compartment of untreated bovine corneal fibroblasts [13]. The relative proportions of CS and HS were found to be 20% and 10%, respectively. Figure 2B (lane C) shows an electrophoretic separation of intracellular GAGs isolated from control cells. The Alcian Blue-stained bands on electrophoretic strips could be characterized by digestion with the chondroitinases and heparin lyase III (Fig. 2C). In addition, we observed in radiolabeling studies that $^{35}SO_4^{2-}$-labeled GAGs of control and treated fibroblasts (tilorone 3 and 20 μM, Cl-90.100 3 μM) could be completely depolymerized by chondroitinase ABC and subsequent incubation with HNO_2 [12]. Taken together, these findings suggest that DS, CS, and HS are the GAGs predominantly produced by bovine corneal fibroblasts in culture, which is in line with previous reports [13, 17, 18]. Therefore, no further qualitative analysis was performed. Kevatan sulfate, although being an important GAG of the corneal stroma *in vivo*, is not synthesized by bovine corneal fibroblasts under cell culture conditions [17, 18].

INTRACELLULAR GAG STORAGE. After treatment of cultured bovine fibroblasts with various concentrations of the four bis-basic compounds, the intracellular GAGs were isolated, separated by cellulose acetate electrophoresis, and quantified by densitometric scanning of Alcian Blue-stained bands.

In the present study, tilorone was found to induce accumulation of intracellular DS and HS in a concentration-dependent manner (Fig. 2A and B). The dose-response curves showed that the range of concentrations that induced either maximum DS storage or maximum HS storage could be separated. DS was the GAG stored predominantly at concentrations $\leq$ 5 μM, whereas the intracellular amount of HS increased markedly at higher drug concen-

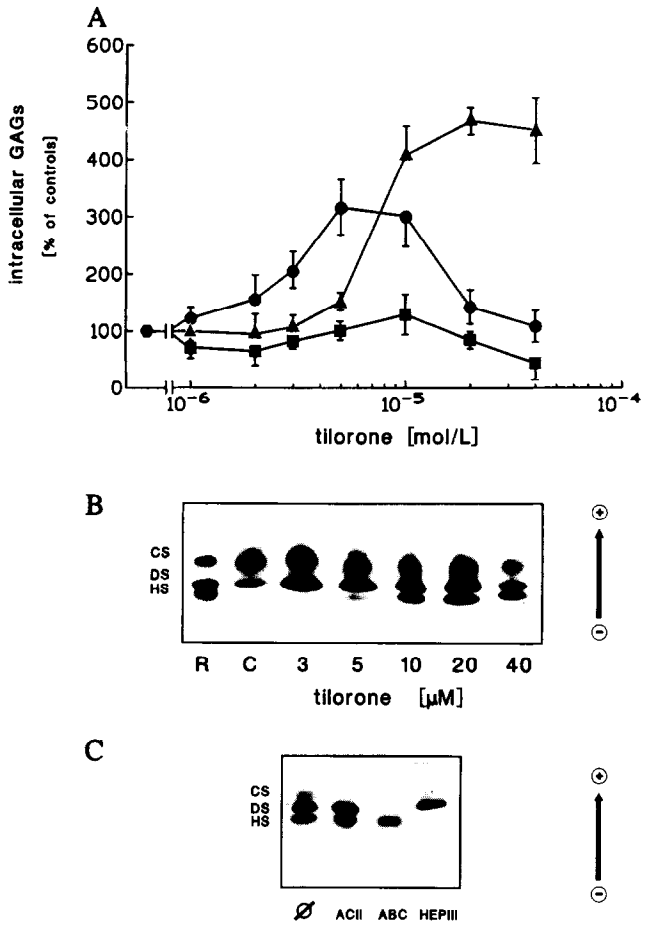

FIG. 2. Bovine corneal fibroblasts; tilorone. Concentration-dependent effects of tilorone on the intracellular levels of DS (●), CS (■), and HS (▲). The fibroblasts were exposed to the indicated concentrations of tilorone for 96 hr. (A) Dose-response curves (mean ± SEM, N = 4–9); intracellular GAGs were quantified after electrophoresis by means of densitometric scanning of Alcian Blue-stained bands and expressed as percentage of untreated controls; (B) Representative electrophoretic separations of intracellular GAGs of controls (lane C) and cultures treated with the indicated concentrations of tilorone. The separation of reference GAG HS, DS, and CS is depicted in the left lane (R). (C) Enzymatic degradation of intracellular GAGs isolated from tilorone-treated (20 μM) fibroblasts. The different lanes indicate the GAGs incubated with control buffer of chondroitin lyase (∅), chondroitin lyase ACII (ACII), chondroitin lyase ABC (ABC), and heparin lyase III (HepIII). The separations of GAGs incubated with the other control buffers are not shown.

trations. Concerning CS, no statistically significant changes could be observed. At concentrations above 20 μM tilorone, the dose-response curves leveled off (HS) or revealed decreasing intracellular galactosaminoglycan content (DS, CS) because of nonspecific toxicity.

The acridine derivative CL-90.100 induced storage of DS and HS, whereas CS accumulation could not be observed (Fig. 3). A pronounced DS storage was induced at concentrations ≤ 4 μM, whereas a statistically significant HS storage occurred at concentrations ≥ 4 μM. When compared with the effects of tilorone, the dose-response

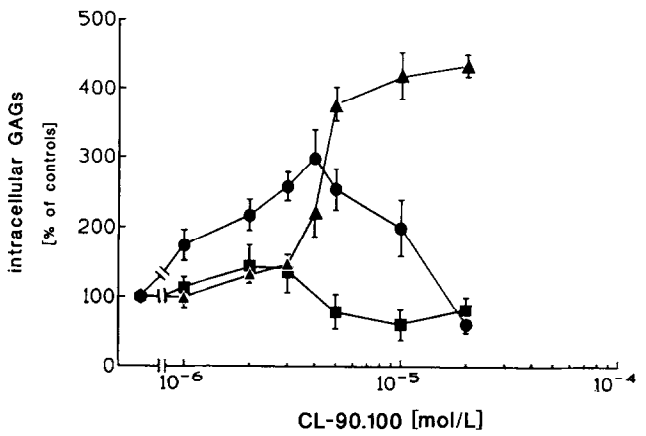

FIG. 3. Bovine corneal fibroblasts; CL-90.100. Dose-response curves for intracellular DS (●), CS (■), and HS (▲) accumulation. Fibroblasts were exposed for 96 hr to the acridine derivative. The depicted curves are based on densitometric scans of electrophoretically separated GAG bands (Alcian Blue stain). The indicated values represent means ± SEM (*n* = 5–9).

curves for storage of DS and HS were shifted to lower concentrations. Treatment with the anthraquinone derivative RMI-10.024 and the fluorene derivative RMI-10.233 (Figs. 4 and 5) caused GAG storage with patterns similar to those evoked by CL-90.100 and tilorone. Again, HS accumulation occurred at higher concentrations than did DS accumulation. RMI-10.233 could not be used in concentrations higher than 10 μM because of its toxicity. The enzymatic digestion of intracellular GAGs after treatment with CL-90.100, RMI-10.024, and RMI-10.233 (data not shown) yielded results identical to those shown for tilorone (Fig. 2C).

At low concentrations of tilorone (≤ 5 μM), CL-90.100 (≤ 4 μM), RMI-10.024 (≤ 5 μM), and RMI-10.233 (≤ 5 μM), intracellular DS content was increased by factors of

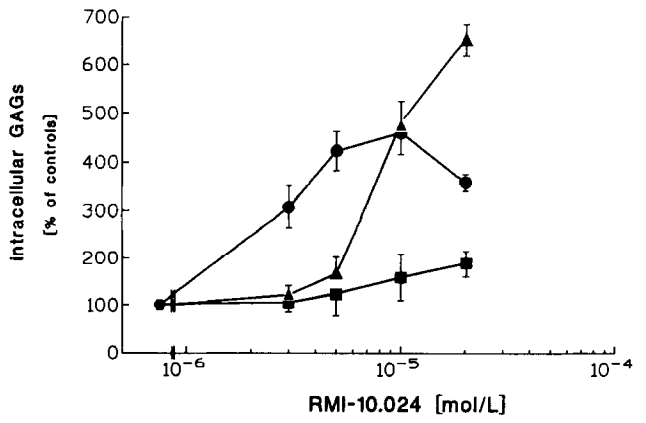

FIG. 4. Bovine corneal fibroblasts; RMI-10.024. Concentration-dependency of intracellular GAG accumulation [DS (●), CS (■), HS (▲)] after treatment (96 hr) with the anthraquinone derivative. The data were obtained by densitometric scanning of Alcian Blue-stained cellulose acetate strips; mean ± SEM, *n* = 3–5.

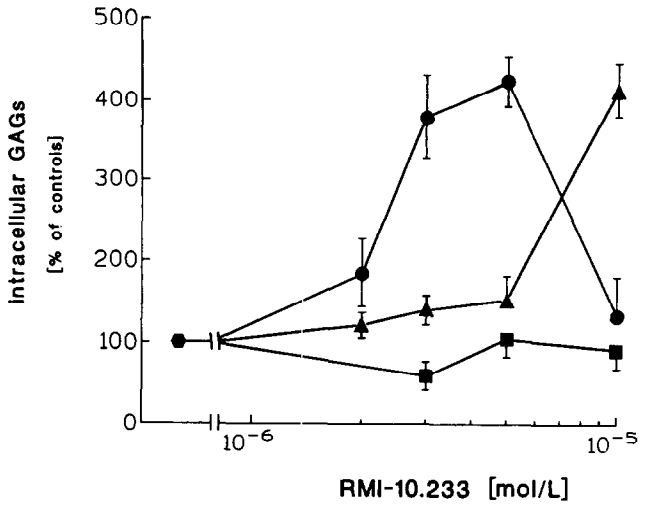

FIG. 5. Bovine corneal fibroblasts; RMI-10.233. Concentration-dependent effects of the fluorene derivative on the accumulation of intracellular DS (●), CS (■), and HS (▲) in cultured bovine fibroblasts. The data were obtained after electrophoretic separation of GAGs on cellulose acetate strips and subsequent densitometric scanning (Alcian Blue stain); mean ± SEM, n = 3–5.

3–4, whereas HS levels were hardly elevated. Taking into account that, in control cultures, 70% of intracellular GAGs consist of DS, it could be calculated that, at low drug concentrations, the accumulated GAGs consisted predominantly of DS (> 90%).

EXTRACELLULAR DS LEVELS AFTER DRUG TREATMENT. In a previous study, it was shown that treatment of bovine corneal fibroblasts with 5 μM tilorone (96 hr) does not affect the amount of secreted DS [13]. This was also demonstrated in the present study for 3 μM CL-90.100 (99 ± 16%) and 3 μM RMI-10.024 (115 ± 14%), suggesting that the pronounced drug-induced DS storage was, at least at low concentrations, not due to disturbed secretion of DS-PGs into the culture medium.

The finding that tilorone-induced DS storage started to decline at concentrations higher than 10 μM (Fig. 2A) raised the question as to whether or not decreased synthesis of DS-PGs might diminish the extent of DS accumulation. This question was addressed by quantification of the amount of extracellular DS at the end of the incubation (96 hr) with 20 μM tilorone. The amount of extracellular DS was found to be decreased to 54 ± 9% (mean ± SEM, N = 4) compared with untreated controls, suggesting that the secretion and/or synthesis of DS was impaired under this condition.

Human Desmodontal Fibroblasts

In cultured human desmodontal fibroblasts, DS accounted for 53 ± 5%, CS for 30 ± 3%, and HS for 17 ± 4% of total intracellular GAGs (mean ± SEM, N = 4). In the extracellular compartment, DS and CS were found to be the only constituents. The pericellular GAG pool was represented mainly by HS and CS with only minor amounts of DS (Fig. 6A). In addition to the results of electrophoretic separation, the identity of GAGs was proved by enzymatic digestion of CS by chondroitin AC II lyase, of DS and CS by chondroitin ABC lyase, and of HS by heparin lyase III. The results of the enzymatic depolymerization of intracellular GAGs are shown in Fig. 6B. The observation that the CS band on the electrophoresis was not completely degraded by chondroitin AC II lyase suggests that it consisted of CS/DS copolymers, which have also been found in bovine corneal fibroblasts and in many other cell types [13, 19–21]. The present characterization of GAGs corresponds to the proteoglycans produced by human peridontal fibroblasts [22].

Human desmodontal fibroblasts were treated (96 hr) with 5 and 20 μM tilorone. The intracellular contents of HS, DS, and CS were found to be elevated after treatment with 5 μM and 20 μM tilorone (Fig. 7). Interestingly, as in the bovine fibroblasts, DS was the GAG accumulating to highest levels after treatment with 5 μM tilorone. Because elevation of the tilorone concentration to 20 μM led only to a small, statistically nonsignificant, increase of DS accumulation, it may be assumed that the storage of DS had already reached maximum values at 5 μM. In contrast, HS storage reached its highest values (7-fold accumulation) at 20 μM. CS was accumulated only 2-fold at either tilorone concentration.

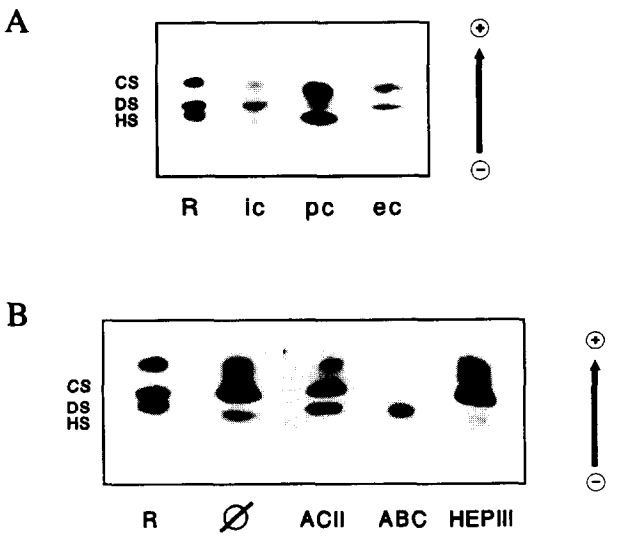

FIG. 6. Human desmodontal fibroblasts. (A) Cellulose acetate electrophoresis of GAGs isolated from the intracellular (ic), pericellular (pc), and extracellular (ec) compartments. The left lane shows the positions of reference GAGs (R). (B) Electrophoretical separation of enzymatically digested GAGs isolated from the intracellular compartment of human peridontal fibroblasts after treatment with 5 μM tilorone. The different lanes indicate electrophoresis after incubation with control buffer of the chondroitin lyase ACII digestion (∅), chondroitin lyase ACII (ACII), chondroitin lyase ABC (ABC), and heparin lyase II (HepIII). The electrophoresis of GAGs incubated with the other control buffers is not shown.

To compare the effects of tilorone with those of ammonia, which is known to cause enhanced secretion of lysosomal enzymes [23, 24], the human fibroblasts were treated with 10 mM NH_4Cl for 96 hr. Under this condition, the distribution pattern of accumulated GAGs was different from that seen with tilorone (Fig. 7). HS storage (5-fold accumulation) was more pronounced than DS storage (3-fold accumulation), which was, in turn, weaker than DS storage after treatment with 5 and 20 μM tilorone, emphasizing the selectivity of tilorone-induced DS accumulation. These data are in line with previous results obtained in cultured bovine fibroblasts after treatment with ammonia [13].

DISCUSSION

Although the details of the pathomechanism of drug-induced mucopolysaccharidosis are still under discussion, there is no doubt that drug-induced GAG storage is due to impaired lysosomal degradation of GAGs [14, 15]. Lysosomal trapping of these compounds is proposed to be a prerequisite for inhibition of lysosomal GAG catabolism. After drug accumulation in acidic compartments, which is driven by the protonization of the two basic nitrogen atoms located in the side chains, millimolar concentrations of drugs were shown to be reached within the lysosomes [13, 25]. In previous studies, lysosomal drug accumulation for tilorone was demonstrated by quantification of drug uptake [13] and for CL-90.100 by fluorescence microscopy [11]. Histochemical examination of cultured cells treated with low concentrations (≤ 5 μM) of these compounds proved that the accumulation of GAGs takes place within the lysosomes. This leads to characteristic storage lysosomes

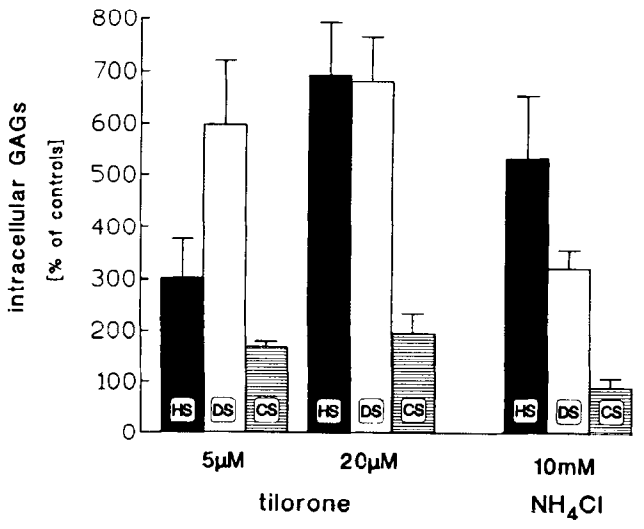

FIG. 7. Human desmodontal fibroblasts. Effect of tilorone (5 and 20 μM) and NH_4Cl (10 mM) on the intracellular levels of HS, DS, and CS. The data were obtained by means of densitometric scanning of electrophoretic separations (Alcian Blue stain) and expressed as the percentage of untreated controls; mean ± SEM, N = 4.

that resemble those in patients with inherited mucopolysaccharidosis (MPS) [8]. An important finding of the present study is that all investigated drugs induced selective DS storage at the same concentrations that induce typical MPS-like cytological lesions. Therefore, it can be concluded that storage lysosomes contain mainly undegraded DS molecules.

A previous study revealed a concentration-dependent increase of GAG storage, expressed in terms of total accumulation of ^{35}S-labeled macromolecules. To further characterize the dose-dependency of drug-induced GAG storage, the question as to whether or not the pattern of stored GAGs changes with rising concentrations was addressed in the present investigation. It turned out that drug-induced GAG storage was characterized by a concentration-dependent shift of the GAG pattern. At high drug concentrations, marked HS storage was observed, in addition to DS storage. Because it had been demonstrated in a previous study that the secretion of lysosomal proenzymes was increased at tilorone concentrations > 10 μM [15], it is conceivable that the loss of lysosomal enzymes might be responsible for the additional HS accumulation in the presence of high drug concentrations.

With regard to structure-activity relationships, the comparison of the investigated compounds, which differed only in their central ring systems, failed to detect important differences concerning the pattern of stored GAGs and the concentration-dependent shift of the GAG pattern. The dose-response curves for the acridine derivative CL-90.100 were shifted merely to lower concentrations compared with the remaining three compounds. This higher potency might be a consequence of higher intralysosomal accumulation ratios in the case of the acridine derivative because of the additional nitrogen atom located in the central ring system.

The analysis of GAGs stored in tilorone-treated human fibroblasts indicates that the prevalent DS storage is not species-dependent. In human cells, as well, low drug concentrations induce storage predominantly of DS, whereas marked HS storage appears with increased drug concentrations. In contrast to the results obtained with bovine fibroblasts, a weak storage of CS could be observed in the case of the human fibroblasts.

The pathomechanism responsible for drug-induced GAG storage is still unknown. It has been proposed that the disturbance of the mannose-6 phosphate receptor-mediated targeting of lysosomal proenzymes that results in a decrease in intralysosomal enzyme activity is responsible [14]. This mechanism was disproved by the finding that secretion of lysosomal enzymes was unchanged at low concentrations (5 μM), inducing the specific morphological alterations and marked DS storage [15]. Another hypothesis proposes formation of complexes between dicationic drug molecules and polyanionic GAGs that would resist the action of lysosomal glycosidases [6, 7]. This hypothesis was supported by the finding of strong physicochemical interactions be-

tween tilorone and DS at concentrations that were probably reached in the lysosomes under our experimental conditions [13]. HS revealed a much weaker interaction and CS did not interact with the tilorone molecules [13]. The affinity of DS for the dicationic molecules is believed to depend on its carbohydrate composition and its secondary or tertiary structures. It is obvious that, in different cell types or tissues, the affinity of DS, CS, and HS for the dicationic drugs may differ, because it is known that the amount and position of sulfate groups, as well as the degree of epimerization of uronic acids, are highly variable. This structural variability might explain why CS also accumulated in human fibroblasts and why HS accumulation was higher than in bovine cells.

The finding of a lower CS and HS accumulation as compared with DS accumulation at low drug concentrations could, theoretically, be explained by a lower turnover of HS and CS. In the case of HS, this assumption is unlikely, because HS accumulated 5-fold over control levels after treatment with high drug concentrations. In the case of CS, a lower turnover could, indeed, be involved, although the NMR-studies [13] suggest that a weaker interaction between dicationic drug molecules and CS is probably the reason for the weak CS storage.

The most important outcome of the present study is that tilorone and three other compounds, that were, like tilorone, characterized by planary aromatic ring systems symmetrically substituted with basic side chains, all induce selective DS storage at low concentrations. This observation suggests that DS, in general, has a higher affinity for these dicationic molecules, which possibly renders the lysosomal degradation of DS in the presence of the dicationic molecules more vulnerable than that of HS and CS.

References

1. Hascall VC, Heinegard DK and Wight TN, Proteoglycans, Metabolism and pathology. In: *Cell Biology of Extracellular Matrix* (Eds. Hay ED) pp. 149–175. Plenum Press, New York, 1991.
2. Chandra P and Wright GJ, Tilorone hydrochloride: the drug profile. *Top Curr Chem* **72:** 125–148, 1977.
3. Angier RB, Citarella RV, Damiami M, Fabio PF, Fields TL, Kang SM, Lin Y, Linch HF, Murdock KC, Petty SR, Wilkinson RG and Lang SA, Synthesis of 3,6-bis(amino-alkoxy)acridines and their effect on the immune system. *J Med Chem* **26:** 1710–1715, 1983.
4. Regelson W, The biological activity of the synthetic polyanion, pyran copolymer (Diveema, MVE, 46015) and the heterocyclic bis DEAE fluorenone derivative, tilorone and congeners: clinical and laboratory effects of these agents as modulators of host resistance. *Pharmac Ther* **15:** 1–44, 1981.
5. Hein L and Lüllmann-Rauch R, Mucopolysaccharidosis and lipidosis in rats treated with tilorone analogues. *Toxicology* **58:** 145–154, 1989.
6. Grave S, Lüllmann H, Lüllmann-Rauch R, Osterkamp G and Prokopek M, Induction of mucopolysaccharidosis in rats by treatment with immunostimulatory acridine derivatives. *Toxicol Appl Pharmacol* **114:** 215–224, 1992.
7. Prokopek M, The tilorone-induced mucopolysaccharidosis in rats. *Biochem Pharmacol* **42:** 2187–2191, 1991.
8. Burmester J, Handrock K and Lüllmann-Rauch R, Cultured corneal fibroblasts as a model system for the demonstration of drug-induced mucopolysaccaridosis. *Arch Toxicol* **64:** 291–298, 1990.
9. Lüllmann-Rauch R and Ziegenhagen M, Drug-induced lysosomal storage of sulfated glycosaminoglycans in cultured bovine and human fibroblasts. *Virchows Arch [Zellpathol]* **60:** 99–104, 1991.
10. Handrock K, Lüllmann-Rauch R and Vogt R, Drug-induced lysosomal storage of sulfated glycosaminoglycans. Studies on the underlying structure activity relationships. *Toxicology* **85:** 199–213, 1993.
11. Handrock K, Laschke A, Lüllmann-Rauch R, Vogt RD and Ziegenhagen M, Lysosomal storage of sulfated glycosaminoglycans in cultured fibroblasts exposed to immunostimulatory acridine derivatives. *Toxicol Appl Pharmacol* **114:** 204–214, 1992.
12. Fischer J, Degradation of free galactosaminoglycan chains is inhibited by two immunomodulatory compounds in cultured fibroblasts. *Naunyn Schmiedebergs Arch Pharmacol* **353,** Supp R78, 1996.
13. Fischer J, Tilorone-induced lysosomal storage of glycosaminoglycans in cultured corneal fibroblasts: Biochemical and physicochemical investigations. *Biochem J* **312:** 215–222, 1995.
14. Gupta DK, Gieselmann V, Hasilik A and von Figura K, Tilorone acts as a lysosomotropic agent in fibroblasts. *Hoppe Seyler's Z Physiol Chem* **365:** 859–866, 1984.
15. Lüllmann-Rauch R, Pods R and Von Witzendorff B, Tilorone-induced lysosomal storage of sulfated glycosaminoglycans can be separated from tilorone-induced enhancement of lysosomal enzyme secretion. *Biochem Pharmacol* **49:** 1223–1233, 1995.
16. Wessler E, Analytical and preparative separation of acidic glycosaminoglycans by electrophoresis in barium acetate. *Anal Biochem* **26:** 439–444, 1968.
17. Bleckmann H and Kresse H, Beeinflussung der Glycosaminoglycansynthese von kultivierten Stromazellen aus Rindercorneae durch Variation dur Kulturbedingungen. *Albrecht v Graefes Arch klin exp Ophthal* **210:** 291–300, 1979.
18. Bleckmann H and Kresse H, Glycosaminoglycan metabolism of cultured cornea cells derived from bovine and human stroma and from bovine epithelium. *Exp Eye Res* **30:** 469–479, 1980.
19. Habuchi H, Yamagata T, Iwata H and Suzuki S, The occurrence of a wide variety of dermatan sulfate-chondroitin sulfate copolymers in fibrous cartilage. *J Biol Chem* **248:** 6019–6028, 1973.
20. Yue BY, Baum JL and Silbert JE, The synthesis of glycosaminoglycans by cultures of rabbit corneal endothelial and stroma cells. *Biochem J* **158:** 567–573, 1976.
21. Axelsson I and Heinegard D, Fractionation of proteoglycans from bovine corneal stroma. *Biochem J* **145:** 491–500, 1975.
22. Larjava H, Häkkinen L, Rahemtulla F, A biochemical analysis of human peridontal tissue proteoglycans. *Biochem J* **284:** 267–274, 1992.
23. Seglen PO, Inhibitors of lysosomal function. *Meth Enzymol* **96:** 737–764, 1983.
24. Gonzalez-Noriega A, Grubb JH, Talkad V and Sly WS, Chloroquine inhibits lysosomal enzyme pinocytosis and enhances lysosomal enzyme secretion by impairing receptor recycling. *J Cell Biol* **85:** 839–853, 1980.
25. MacIntyre AC and Cutler DJ, The potential role of lysosomes in tissue distribution of weak bases. *Biopharm Drug Dispos* **9:** 513–526, 1988.