Cancer Chemother Pharmacol (1992) 30: 483–486

Cancer Chemotherapy and Pharmacology

Short communication

Inhibition of growth factor binding and intracellular Ca^{2+} signalling by dextran sulfates of different sizes and degrees of sulfation*

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Received 17 December 1991/Accepted 15 April 1992

Summary. The ability of dextran sulfates of varying molecular sizes (5–500 kDa) and degrees of sulfate substitution (0.3–1.9) to inhibit the binding of platelet-derived growth factor (PDGF) to intact Swiss 3T3 fibroblasts and to inhibit inositol(1,4,5)trisphosphate-dependent release of Ca^{2+} in permeabilized Swiss 3T3 cells was examined in the present study. Significant correlations were found between increased molecular size of the dextran sulfates and inhibition of both PDGF binding ($r = 0.77$) and Ca^{2+} release ($r = 0.72$). The degree of sulfate substitution did not correlate with inhibition of either activity.

Introduction

Attention has recently focused on the polysulfated antitrypansomal drug suramin as a potential agent for the treatment of cancer [21, 31]. Clinical trials of suramin have demonstrated activity against some human cancers [1, 12, 31]. The ability of suramin to inhibit cell growth is frequently ascribed to an extracellular action involving blockade of the binding of growth factors to their cell-surface receptors [2, 10, 18, 20, 34]. Suramin is also taken up by cells [11, 19] and may exert intracellular actions. For example, we have shown that suramin inhibits phosphoinositide-specific phospholipase C and blocks the release of intracellular Ca^{2+} by inositol(1,4,5)trisphosphate [Ins(1,4,5)P_3], two key steps in intracellular signalling [24, 29]. Other polysulfated compounds with diverse structures, including heparin, dextran sulfate, and azo dyes, also block growth-factor-receptor binding and the Ins(1,4,5)P_3-mediated release of intracellular Ca^{2+} [24, 28, 38]. High-molecular-weight heparin and dextran sulfate have been reported to be more effective than low-molecular-weight forms as inhibitors of Ins(1,4,5)P_3-mediated Ca^{2+} release [28, 32].

We attempted to study the relationship between inhibition by polysulfated compounds of the apparently diverse activities of growth-factor-receptor binding and intracellular Ca^{2+} release using a series of dextran sulfates of varying molecular size and degrees of sulfate substitution. We found a decreasing potency for the inhibition of both growth factor-receptor binding and intracellular Ca^{2+} release with decreasing molecular size of the dextran sulfates. It was not possible to separate the ability of the dextran sulfates to inhibit the two activities. Within the range studied, the degree of sulfate substitution had no effect on either growth-factor-receptor binding or intracellular Ca^{2+} release.

Materials and methods

The dextran sulfates were kindly supplied by Dr. T. de Belder, Pharmacia AB (Uppsala, Sweden). The term DS represents the number of substituents per glucose unit. The maximal DS possible for dextran is 3, as it contains 3 hydroxyl groups per glucose unit. The molecular weights and DS values for the dextran sulfates used were 5 kDa, DS 0.3; 12 kDa, DS 1.8; 21 kdA, DS 1,8; 22 kDa, DS 1.2; 76 kDa, DS 0.7; 76 kDa, DS 0.4; 116 kDa, DS 1.2; and 120 kDa, DS 1.7. Dextran sulfate 500 kDa (DS 1.9) and heparin were obtained from Sigma Chemical Co. (St. Louis, Mo.). Ins(1,4,5)P_3 was supplied by Calbiochem (Irvine Calif.), and $^{45}CaCl_2$ (23 mCi/mg) and (s-*sis*)-[^{125}I]-PDGF (780 Ci/mmol) were purchased from Amersham Corp. (Arlington Heights, Ill.). Swiss 3T3 fibroblasts were obtained from the American Tissue Culture Collection (Rockville, Md.) and were grown as described elsewhere [28].

$^{45}Ca^{2+}$ uptake and $^{45}Ca^{2+}$ release by Ins(1,4,5)P_3 from the adenosine triphosphate (ATP)-dependent nonmitochondrial stores of saponin-permeabilized Swiss 3T3 fibroblasts were measured as previously described [28]. Preliminary studies showed that ATP-dependent $^{45}Ca^{2+}$ uptake by the cells was maximal at 6 min. At 6.25 min, 10 μM Ins(1,4,5)P_3 was added and the $^{45}Ca^{2+}$ remaining in the cells at 7 min was measured and expressed as a percentage of that found in the cells at 6 min. Dextran sulfates were added to the incubations at 0 min. The binding of 3.3×10^{-11} M [^{125}I]-PDGF to intact Swiss 3T3 fibroblasts was measured over

* Supported by NIH grant CA 42286

Abbreviations: DS, degree of substitution; PDGF, platelet-derived growth factor; IC_{50}, concentration giving 50% inhibition of response

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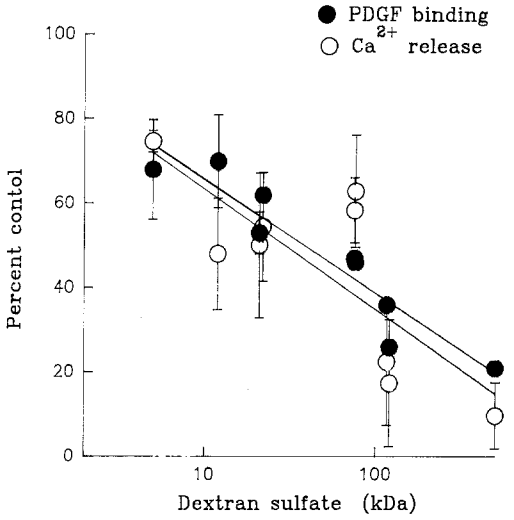

Fig. 1. Ins(1,4,5)P_3-mediated Ca^{2+} release by high-molecular-weight dextran sulfates; 10 μM Ins(1,4,5)P_3 was used to release $^{45}Ca^{2+}$ from the nonmitochondrial stores of saponin-permeabilized Swiss 3T3 fibroblasts in the presence of dextran sulfate at 500 (○) or 120 kDa (●). Data represent mean values for 5 determinations, and bars indicate the SD

Table 1. Dextran sulfate inhibition of Ca^{2+} release and [^{125}I]-PDGF receptor binding in Swiss 3T3 fibroblasts

Dextran sulfate mol. wt. (kDa)	DS	$^{45}Ca^{2+}$ release (% control)	[^{125}I]-PDGF binding (% control)
5	0.3	75 ± 2	68.8 ± 11.8
12	1.8	48 ± 13	70.1 ± 11.2
21	1.8	50 ± 13	53.0 ± 5.1
22	1.2	54 ± 13	62.1 ± 1
76	0.4	63 ± 13	46 ± 2
76	0.7	58 ± 8	47 ± 0
116	1.2	22 ± 15	36 ± 0
120	1.7	17 ± 15	26 ± 6
500	1.9	10 ± 8	21 ± 2

The release of $^{45}Ca^{2+}$ by 10 μM Ins(1,4,5)P_3 from the nonmitochondrial stores of saponin-permeabilized Swiss 3T3 fibroblasts was measured using dextran sulfates at 25 μg/ml. The binding of [^{125}I]-PDGF to intact Swiss 3T3 fibroblasts was determined using dextran sulfates at 100 μg/ml. Data represent mean values ± SD obtained for the appropriate control in the absence of dextran sulfate (control values: $^{45}Ca^{2+}$ release, 90.4 ± 5 pmol/10^6 cells; [^{125}I]-PDGF binding, 13.1 ± 2.3 fmol/10^6 cells DS, Degree of substitution of the dextran sulfate.

4 h at 4° C as described by Bowen-Pope and Ross [4]. The growth of Swiss 3T3 cells on plastic surfaces was determined over 7 days [23].

Results

Inhibition of $^{45}Ca^{2+}$ release

The dextran sulfates had no effect on the ATP-dependent uptake of $^{45}Ca^{2+}$ by saponin-permeabilized Swiss 3T3 fibroblasts (data not shown). All dextran sulfates inhibited the Ins(1,4,5)P_3-dependent release of $^{45}Ca^{2+}$ from nonmitochondrial stores of saponin-permeabilized Swiss 3T3 fibroblasts. Figure 1 shows the concentration-response curves generated for the inhibition of Ins(1,4,5)P_3-mediated Ca^{2+} release by the two most potent dextran sulfates. The mean (± SE) concentrations producing 50% inhibition (IC_{50}) of Ca^{2+} release were 10.9 ± 2.4 μg/ml for the 500-kDa dextran sulfate and 18.3 ± 6.0 μg/ml for the 120-kDa dextran sulfate. All of the dextran sulfates were inhibitors of $^{45}Ca^{2+}$ release (Table 1). A correlation was found ($r = 0.72$, $P < 0.05$) between the inhibition of $^{45}Ca^{2+}$ release and the increasing molecular weight of the dextran sulfates. The DS of the dextran sulfates showed no significant correlation with the inhibition of $^{45}Ca^{2+}$ release.

Inhibition of [^{125}I]-PDGF binding

The binding of [^{125}I]-PDGF to Swiss 3T3 fibroblasts was inhibited by the dextran sulfates (Table 1). A significant correlation was observed ($r = 0.77$, $P < 0.05$) between the inhibition of [^{125}I]-PDGF binding and the increasing molecular weight of the dextran sulfates. The DS of the dextran sulfates did not affect the inhibition of [^{125}I]-PDGF binding. Inhibition of both $^{45}Ca^{2+}$ release and [^{125}I]-PDGF binding by the dextran sulfates of different molecular weights showed a highly significant positive correlation ($r = 0.82$, $P < 0.01$).

Cell proliferation

At concentrations of up to 750 μg/ml dextran sulfate (500 kDa) did not inhibit the growth of Swiss 3T3 fibroblasts. The glycosoaminoglycose heparin, which served as a positive control, inhibited Swiss 3T3 fibroblast growth, with the mean IC_{50} value being 83 ± 3 μg/ml.

Discussion

The recent interest in suramin as an antitumor agent has focused attention on polysulfated compounds as inhibitors of cell proliferation. A number of polysulfated compounds, including dextran sulfates [7, 20, 26], heparin [22, 25, 33, 37], pentosan polysulfate [9, 35], and suramin [2, 6, 10], have been reported to inhibit the growth of cells in culture. Dextran sulfate and heparin are generally less effective inhibitors of cell growth than is either suramin or pentosan sulfate. It is not known whether is a common mechanism exists for cell-growth inhibition by the polysulfated compounds. Dextran sulfate, heparin, suramin, and pentosan polysulfate have been reported to inhibit the binding of growth factors to cell-surface receptors [8, 28, 29, 33, 35]. However, these agents also exert intracellular actions, including the inhibition of intracellular Ca^{2+} release in permeabilized cells [28, 29, 32] and the inhibition of protein kinase C [17, 27, 37]. It is therefore possible that the varying abilities of polysulfated compounds to inhibit cell growth may be related to differences in their intracellular actions as well as to differences in their extracellular effects on growth factor-receptor binding. This possibility

prompted us to study the ability of a series of dextran sulfates of different molecular sizes and degrees of sulfation to inhibit PDGF receptor binding and Ins(1,4,5)P_3-mediated Ca^{2+} release in an attempt to determine whether the two activities could be separated.

We have previously observed that the ability of both heparin and dextran sulfate to block Ins(1,4,5)P_3-mediated Ca^{2+} release appears to depend on the molecular size of the compound, with a high-molecular-weight form being more active (on a weight basis) than a low-molecular-weight form [28]. In the present study we confirmed that high-molecular-weight dextran sulfate (500 kDa) was an effective inhibitor of both $^{45}Ca^{2+}$ release and [^{125}I]-PDGF binding. Using a series of dextran sulfate analogues of lower molecular weight, we found that the inhibition of $^{45}Ca^{2+}$ release and [^{125}I]-PDGF binding decreased progressively with the decreasing molecular size of the dextran sulfates. The two inhibitory activities were closely correlated and could not be separated.

The glycosoaminoglycose heparin blocks Ins(1,4,5)P_3-mediated intracellular Ca^{2+} release by competing with Ins(1,4,5)P_3 for binding to a specific receptor on the endoplasmic reticulum [13, 36]. Tones et al. [32] have reported that the ability of heparin to block the binding of Ins(1,4,5)P_3 to its endoplasmic reticulum receptor decreases with the decreasing molecular weight of the heparin. It is probable that other polysulfated compounds act in a similar manner by blocking the binding of Ins(1,4,5)P_3 to its receptor [28, 29].Yamamoto et al. [38] reported that a low-molecular-weight dextran sulfate (5 kDa) inhibited the binding of Ins(1,4,5)P_3 to its receptor in permeabilized smooth-muscle cells. Bootman et al. [3] have found that polysulfated azo dyes inhibit Ins(1,4,5)P_3 receptor binding. Sulfation is necessary for the inhibition by heparin of Ins(1,4,5)P_3-mediated Ca^{2+} release [13, 32]. We have previously reported that nonsulfated dextran does not block Ins(1,4,5)P_3-mediated Ca^{2+} release [28]. The Ds of the dextran sulfates we studied did not affect the ability of dextran sulfates to block either Ins(1,4,5)-mediated Ca^{2+} release or [^{125}I]-PDGF-receptor binding. However, considering the limited DS range available to us, it may not have been possible to detect such an effect.

Dextran sulfate (500 kDa) did not block the growth of Swiss 3T3 fibroblasts at concentrations considerably higher than those required for the complete inhibition of PDGF binding. In contrast, suramin is a potent inhibitor of the growth of fibroblasts [24] and other cells [2, 6, 10, 11, 21]. In serum, PDGF is the major growth factor responsible for fibroblast proliferation [16]. However, other growth factors are known to be required for fibroblast proliferation [5], and it is possible that dextran sulfates do not block the binding of other growth factors as effectively as does suramin. Although it is a large, highly charged molecule, suramin is taken up by cells [11, 19]. It may thus exert intracellular actions, for example, the inhibition of intracellular signalling mechanisms [28, 29], that contribute to its cell-growth-inhibitory activity. Pentosan polysulfate has also ben purported to show intracellular growth-inhibitory activity [9]. Dextran sulfate (60 kDa) has been reported to be taken up into the cytoplasm and nucleus of a virally transformed cell [15]. In general, although highly charged, large molecules such as dextran sulfate can be taken up by cells via endocytosis, for example, via the coated-vesicle pathway, they remain largely confined to intracellular vesicles [14, 30]. Thus, the question remains as to whether sufficient amounts of dextran sulfate reach the cytoplasm to inhibit Ins(1,4,5)P_3-mediated Ca^{2+} release in intact cells.

The reason why polysulfated compounds such as dextran sulfates should inhibit such apparently diverse activities as growth-factor-receptor binding and Ins(1,4,5)P_3-mediated Ca^{2+} release remains unclear. Such inhibition is unlikely to be a specific effect of the polysulfated compounds and may be related to their high negative charge. The question as to why a decrease in the molecular size of dextran sulfates should make them less effective inhibitors of these processes also remains unanswered. Possibly, the ability of the higher-molecular-weight dextran sulfates to bind to and immobilize centers of positive change on the receptors and surrounding molecules in the membrane may make them more effective inhibitors.

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