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A SUBSTRATE ANALOG INHIBITOR FOR ARYLSULFATASE REDUCES NK CELL CYTOTOXICITY

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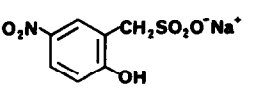
A synthetic aromatic sulfonate (I), which has been found to be an effective inhibitor of arylsulfatase, reduces NK-cell mediated cytotoxicity by ca. 60% at 10 μM concentration. At lower concentrations the effect is concentration dependent, but no further reduction of cytotoxicity is observed at concentrations above 10 μM.

Arylsulfatase hydrolyzes esters of aromatic alcohols and sulfuric acid and is present in most human tissues, with the exception of mature erythrocytes (1, 2). The physiologic and clinical significance of the arylsulfatases has been discussed by several investigators (3, 4, 5). Recently, it has also been suggested that arylsulfatases may be involved in NK-cell mediated cytolysis (6). The weak competitive inhibitors of arylsulfatase Na_2SO_4 and Na_2SO_3 were found to inhibit NK-cell mediated cytotoxicity by approximately 50% (5). Since these inhibitors for arylsulfatases are both non-specific and toxic to cells, they are of only limited usefulness in the study of arylsulfatase in intact cell systems. For this reason a stable structural analog (I) of the standard arylsulfatase substrate, p-nitrocatechol sulfate, was synthesized as a possible selective inhibitor of the enzyme. The synthetic analog I (sodium 2-hydroxy-5-nitro-α-toluenesulfonate) was confirmed to be a good competitive inhibitor of aryl-sulfate sulfohydrolase (E. C. No 3.1.6.1) using a commercial enzyme prepared from abalone. The study of the effect of I at various dose levels on the cytotoxicity of NK cells has strengthened the earlier indications that arylsulfatases may serve as important mediators of NK-cell cytotoxicity.



I

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MATERIALS AND METHODS

Sodium 2-hydroxy-5-nitro-α-toluenesulfonate (I) was synthesized from p-nitrophenol by (a) chloromethylation and (b) displacement of chloride using sodium sulfite (7).

Arylsulfatase (aryl-sulfate sulfohydrolase EC 3.1.6.1) derived from abalone entrails was obtained from Sigma Chemical Co. A K_m of 0.6 mM for p-nitrocatechol sulfate as substrate at 36° and pH 5 was determined with this enzyme by spectrophotometric measurements (410 nm) and Lineweaver-Burk analysis. Using this substrate and inhibitor I at varying concentration a value of K_i of 2.4 mM (36°, pH 5) was derived for the inhibitor from kinetic measurements after plotting the data according to Dixon.[8]

Human NK-cells were purified by discontinuous Percoll-gradient centrifugation as described elsewhere (5, 6). The target melanoma cells were prelabelled with 3H-proline and incubated overnight with NK effector cells in RPMI 1640 medium supplemented with 10% fetal calf serum at 37 °C, 5% CO_2 and 100% humidity. The effector/target cell ratio was 100 : 1. To study the effect of the inhibitor on arylsulfatase activity, NK-cells were preincubated with 0 to 200 μM of inhibitor for 1 hr at 37 °C. The same concentration of inhibitor was maintained after addition of the NK-cells to the target cells. Cytotoxicity was measured as described before (6).

RESULTS

The synthetic arylsulfonate I is an effective competitive inhibitor of hydrolysis (K_i 2.4 mM) of p-nitrocatechol sulfate (K_m 0.6 mM) by arylsulfatase from abalone entrails.

As seen in Fig. 1, incubation with the competitive inhibitor for arylsulfatase (I) reduced NK-cell mediated cytotoxicity. A maximum of 60% inhibition was observed with 10 μM of inhibitor. Inhibition of cytotoxicity appeared to be dose dependent. Control cultures showed that neither the effector NK-cells nor the target melanoma cells were adversely affected, even when incubated with 500 μM of the inhibitor for 20 hr. In previous studies (6) it was shown that NK-cell cytotoxicity was reduced up to 50% by sodium sulfate or sodium sulfite at milli molar concentrations, and it was proposed that this reduction is due to inhibition of arylsulfatase activity. In the earlier work studies on dose-dependence had not been possible because of the non-specific toxic effect of sodium sulfate or sulfite on cells.

DISCUSSION

Despite a large number of published studies on the subject of sulfatase enzymes relatively little is known about the role of these proteins in biological systems. Sulfate esters of such non-polymeric substances as steroids, lipids, and oligosaccharides are synthesized by the action of sulfotransferase enzymes on the appropriate substrates. In addition, sulfate ester biosynthesis is a common biochemical process for the detoxification of noxious substances such as phenols. However, the role of sulfatases probably extends

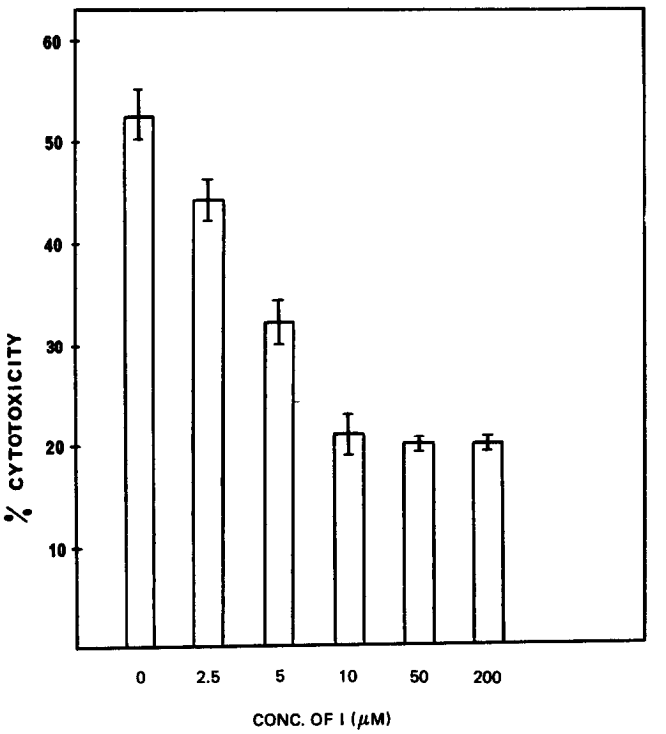

Fig. 1. Inhibition of NK-cell cytotoxicity by sodium 2-hydroxy-5-nitro-α-toluenesulfonate (I) as a function of concentration.

beyond their action to remove sulfate from such substrates. A number of serious inherited metabolic diseases are known which are characterized by sulfatase deficiency, for example, metachromic leukodystrophy (9, 10). Elevated activities of arylsulfatases have been observed in other disease states (11, 12) but the significance of such findings are unclear.

On the other hand, arylsulfatases appear to play some role in NK-cell mediated cytolysis (6), and it is thus possible that the removal of anionic groups by this enzyme have physiologic or pathologic effects not yet recognized. In order to gain further insight into the function of this enzyme in a physiologic or clinical setting, the availability of a specific, non-toxic inhibitor is invaluable.

Using the synthetic inhibitor I, we have shown that at a concentration of 10 μM, 60% of NK-cell mediated cytolysis could be inhibited without affecting the cell viability. There was dose dependence, but no further inhibition was noted beyond this concentration. These results with I strengthen the case for arylsulfatase involvement in NK-cell cytotoxicity.

The use of synthetic analogs of sulfate esters in which the oxygen link between carbon and sulfur is replaced by methylene may be a valuable tactic in other investigations of the role of arylsulfatases in biological systems.

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