Fluorometric Assay of α-L-Iduronidase in Serum for Detection of Affected and Carrier Animals in a Canine Model of Mucopolysaccharidosis I

Robert M. Shull and Nancy E. Hastings

We investigated measuring serum α-L-iduronidase (EC 3.2.1.76) by a sensitive fluorometric assay in 28 members of a canine family with mucopolysaccharidosis I. If assayed on the day of collection, serum was an acceptable specimen for identifying affected, carrier, and normal dogs. The overall correlation (r) between iduronidase activity in serum and mononuclear leukocytes was 0.966. However, iduronidase was extremely labile in serum refrigerated or frozen for 48 h.

Additional Keyphrases: animal models of heritable disorders • enzyme activity • sample handling • variation, source of • serum vs mononuclear leukocytes as sample

The diagnosis of mucopolysaccharidosis I (MPS I; Hurler and Scheie syndromes) has depended upon demonstration of a deficiency of the lysosomal enzyme α-L-iduronidase (EC 3.2.1.76; glucosaminoglycan α-L-iduronohydrolase) since the discovery in 1972 of this enzyme's involvement (1, 2). The most widely used specimens for this determination have been homogenates of cultured fibroblasts or leukocytes from affected individuals or carriers (3). Analyses for iduronidase in leukocytes (4–7) have gained more popularity because they forego the necessity of time-consuming and costly tissue culture; however, they still require isolation of relatively pure populations of leukocytes or mononuclear cells from blood. Leukocyte isolation also is time consuming and presents problems for those sending specimens to laboratories through the mail.

Regardless of specimen used, the first procedures developed involved the hydrolysis of the synthetic substrate phenyliduronide to iduronic acid and phenol, the latter being measured colorimetrically. These procedures were insufficiently sensitive to detect iduronidase activity in serum from healthy, unaffected subjects (3). The advent of fluorometric assays and the substrate 4-methylumbelliferyl-α-L-iduronide resulted in greater sensitivity and shorter assay times, and various modifications of these assays—with leukocytes, fibroblasts, and cultured amniotic cells as samples—are now in use (8–11) for diagnosis of MPS I and identification of heterozygous carriers.

We report our studies with serum as the specimen for detecting heterozygous carriers of MPS I, in which we used a fluorometric assay and sera collected from a research colony of dogs affected with MPS I. These animals have been studied pathologically, biochemically, and genetically, and their disease has been found to be essentially identical to human α-L-iduronidase deficiency (12–14).

Materials and Methods

Serum was isolated from 28 dogs in four generations of a canine MPS I family. The iduronidase activity was assayed on the same day as collection, but we also studied sera that had been refrigerated (5 °C) or frozen (−20 °C) before assay.

Duplicate tubes containing 50 μL of serum and 25 μL of 4-methylumbelliferyl-α-L-iduronide, 2.5 mmol/L in formate buffer (0.4 mol/L, pH 3.5) (supplied by Dr. Elizabeth Neufeld, National Institutes of Health, Bethesda, MD), were incubated in a 25 °C waterbath for 7 h. A reagent blank (50 μL of isotonic saline, NaCl 9 g/L, plus 25 μL of reagent) was also run in duplicate. Serum blanks containing serum and formate buffer were also assayed for each animal. At the end of the incubation period, the reaction was halted by adding 1.0 mL of glycine-carbonate buffer (0.5 mol/L, pH 9.88), after which we measured the fluorescence of the liberated 4-methylumbelliflorene (4-MU) with a Ratio Fluorometer II (Farrand Optical Co., Valhalla, NY). A 1 nmol/L 4-MU standard gave a fluorescence reading of 2.48, from which we calculated the enzyme activity in serum as nanomoles of substrate hydrolyzed per liter of serum per hour of incubation.

For comparison, we also determined iduronidase activity in mononuclear leukocytes collected and isolated at the same time as the serum samples. Blood diluted with an equal volume of isotonic saline was layered onto Ficoll-Hypaque (specific gravity 1.077; Pharmacia Fine Chemicals, Piscataway, NJ), centrifuged at 400 × g for 30 min, then washed twice with isotonic saline. The leukocyte pellets in 0.25 mL of saline were then lysed by repeated freeze-thawing (four times) in a mixture of ethanol/solid CO₂ and sonication in an ice bath. The protein content of the final lysate was determined by the procedure of Lowry et al. (15). The enzyme assay was identical to the procedure with serum samples except that each sample contained 40 μg of protein in 25 μL total volume, and was incubated for 1 h at 37 °C. Iduronidase activity in leukocytes was expressed as nanomoles per milligram of protein per hour of incubation.

Within-run precision was determined for the serum assay in a separate experiment in which sera were analyzed in quadruplicate. The mean, SD, and CV were determined for assays with these sera and for those from all dogs after categorization as homozgyous affected, heterozygous, or normal. We used simple linear regression to evaluate the correlation, if any, between iduronidase activities in serum and leukocytes.

Results

Both assays identified seven dogs as homozygous affected, 15 (tentatively) as heterozygotes, and six as homozgyous normal. Five of the 15 dogs classified as heterozygous have been confirmed as heterozygotes by further breeding trials. Table 1 summarizes the serum and leukocyte enzyme values for the three categories. Both assays detected all seven clinically affected dogs with no iduronidase activity. In neither assay did results overlap for dogs tentatively identified as carriers and for known normal dogs; however, further breeding trials are necessary for certain categorization of some of the dogs. The comparison of all serum and leukocyte values for iduronidase activity is shown in Figure 1. By linear regression the overall coefficient of correlation for serum and leukocyte activities (r) is 0.966 (p < 0.001).
Table 1. α-L-iduronidase Activities in Serum and Leukocyte Samples from a Canine Family with MPS I

<table>
<thead>
<tr>
<th>iduronidase activity</th>
<th>Affected dogs (n = 7)</th>
<th>Heterozygous carriers (n = 15)</th>
<th>Normal dogs (n = 6)</th>
</tr>
</thead>
</table>

**In serum, nmol/L per hour**
- Mean: 0 → 317 → 794
- SD: 0 → 89
- Range: 0 → 357–967

**In leukocytes, nmol/mg protein per hour**
- Mean: 0 → 0.82 → 2.45
- SD: 0 → 0.33
- Range: 0 → 0.41–1.46

![Graph](image.png)

**Fig. 1. Linear regression comparison of serum and leukocyte activities of iduronidase in 28 dogs.** The single dot at the origin represents seven affected dogs with no iduronidase detected by either assay. Five dogs—with serum iduronidase values of 135, 207, 255, 365, and 367 nmol/L per hour—have been proven to be heterozygous by breeding results.

Repetitive analysis of sera from individual normal and heterozygous dogs yielded CVs ranging from 4.14% to 9.61% (mean 6.61%). When paired serum samples from each dog were compared, the duplicate values rarely differed by more than 10%.

The activity of iduronidase in canine serum was very unstable during storage. Eight sera, assayed when fresh and then after having been frozen at −20 °C or refrigerated at 5 °C for 48 h, showed marked enzyme loss—to approximately 30% and 0%, respectively, of their original values.

**Discussion**

The source of iduronidase in normal canine serum is not known, but may be from platelets that are damaged during clotting. Freshly collected plasma contained significantly less iduronidase than did serum (data not shown), which suggests that leukocytes, which contain high concentrations of iduronidase and other lysozyme enzymes, may not be the source of this activity in serum—although the high correlation between iduronidase activity in serum and in concurrently harvested leukocytes makes this an otherwise attractive hypothesis.

Regardless of the source of the enzyme in canine serum, fresh serum clearly is of value in identifying dogs with MPS I and carrier animals. In no instance was there a clinically significant disparity between results for serum and leukocyte samples. When dogs from a single litter (we studied three litters) were ranked in order of their serum and leukocyte enzyme activities, the results were generally the same or differed for only a single animal. Activities of iduronidase in serum also appear to be consistent within individual dogs as shown by ranking littersmates on the basis of serum samples collected three times during a month. Measurement of α-L-iduronidase in serum has now become the standard practice with our colony of dogs as the initial indicator of the enzyme status of a new litter. It is also useful in monitoring response to therapy (bone marrow transplantation).

The true value of iduronidase measurement in serum must, of course, ultimately be assessed in regard to its use in human medicine. If iduronidase in human serum is as labile in vitro as the canine enzyme is, our findings will be of importance to individuals who must send specimens through the mail. Nonetheless, measurement of serum iduronidase should be studied in human specimens because of the ease of the procedure and its potential for use if freshly collected serum is available. Previous colorimetric studies on serum iduronidase in humans (5) may have been affected by a short half-life of the enzyme when stored before analysis, and by the inherent insensitivity of colorimetric assays.

**References**


