The Laboratory Evidence of Efficacy of Hydroxyurea in the Treatment of Sickle Cell Disease

Eugene P. Orringer, M.D.

Since its approval by the FDA in 1996, hydroxyurea (HU), a ribonucleotide reductase inhibitor, has had a major impact on the clinical expression of sickle cell disease (SCD). As the first agent clearly demonstrated to reduce the frequency of such sickle cell-related complications as vaso-occlusive crises and episodes of acute chest syndrome, HU has now been given to many patients, particularly those who are severely affected by SCD. Because of concerns regarding its side effects following long-term exposure as well as its potential as a carcinogen, a mutagen, and/or a teratogen, the initial experience with HU in patients with SCD was limited to adults. Over time, however, when it became apparent that with careful administration and follow-up, HU could be given safely to adult patients with SCD, children also began to receive this agent. Initially, they were given HU as participants in clinical trials, but soon thereafter it also became part of standard therapy for children with SCD. In most cases, children, like adults, have shown substantial benefit following treatment with HU.

Because data from the Cooperative Study of Sickle Cell Disease (CSSCD) indicated that the percentage of fetal hemoglobin (HbF) could influence such manifestations of SCD as the frequency of painful events (1), the occurrence of episodes of acute chest syndrome (2), and overall life expectancy (3), and since HU (and other cytotoxic agents) have been found to enhance HbF production (4-5), it was initially presumed that the beneficial effect of HU was a direct consequence of its ability to increase the percentage of HbF. In the initial multicenter trial in which HU was given to a total of 32 adult patients with SCD who received the drug for a period of at least 16 weeks, a highly significant increase in the mean circulating level of HbF (from 4% to 15%) was observed (6). Similar increases in F-cells, F-reticulocytes, and the amount of HbF per F-cell were also noted in these study subjects. Although not designed nor sufficiently powered to demonstrate HU's clinical benefit in terms of vaso-occlusive episodes, many of the participants in this NIH-funded Phase I safety trial clearly appeared to receive clinical benefit from HU. In addition to the increase in HbF, these study subjects exhibited a number of highly significant changes in a variety of other hematological parameters including increases in total Hgb and MCV and decreases in total WBC, neutrophils, reticulocytes, and platelets. The laboratory results from this Phase I study and the favorable safety profile that was observed in these patients served as the basis for the Phase III Multicenter Study of Hydroxyurea (MSH) (7). While the MSH also showed an HU-induced increase in the various HbF measurements, the most prominent increment in these HbF parameters in the HU-treated patients was seen during the initial three months of therapy. Thereafter, the overall increases in the various HbF measurements trended downward such that by the end of year two, the various HbF parameters

achieved by the HU-treated patients in the MSH were substantially less than the HbF levels that were noted at the end of the earlier, 16 week Phase I study. Two subsequent analyses, in which the results of the 299 participants the MSH were subdivided into quartiles according to their response to HU, found that those patients with the best clinical response in terms of vaso-occlusive crises also had the most robust and sustained increases in both HbF and MCV (8-9). However, these same two analyses also found that those patients in the quartile with the fewest vaso-occlusive events also had the lowest numbers of circulating neutrophils, monocytes, reticulocytes, and platelets. Therefore, it was difficult to be certain which of these various changes (or perhaps what combination of changes) was actually responsible for the clinical efficacy of HU observed in this clinical setting.

In children, the response to HU was first examined in the Pediatric Hydroxyurea Safety Trial, often referred to as HUG-KIDS (10). Much like the initial Phase I adult trial described above, the NIH-funded HUG-KIDS study was not designed to analyze the clinical efficacy of HU in terms of vaso-occlusive crises. Rather, this Phase I/II safety study showed that HU was safe and well-tolerated in children with SCD. In addition, just like the original Phase I study conducted in adults, the children who participated in HUG-KIDS showed a substantial increase in the mean circulating level of HbF (7.3% to 17.8%) during the 12 months of this study. These investigators also found that those children who achieved the highest HbF responses after 12 months of HU therapy: a) had the highest levels of HbF at baseline; and b) were able to tolerate the highest dosages of HU throughout the course of the study. Finally, when the peak HbF response was broken down into quartiles (i.e., maximal to minimal HbF responders), highly significant correlations were observed between the magnitude of the increase in HbF and the extent of: a) the increase in total hemoglobin and MCV; and b) the decrease in total WBC and reticulocytes. In a second pediatric Phase I Safety Study that was conducted at a single institution, virtually identical hematologic results were observed (11). While neither of these two pediatric studies looked at the frequency of painful events and/or hospitalizations, a few other studies did. While these were not randomized, placebo-controlled trials, they did obtain baseline data such that the HU-treated patients served as their own controls. Jayabose et al. (12) treated 14 SCD children with HU and found a highly significant decrease in the number of vaso-occlusive events (both painful crises and episodes of acute chest syndrome) when compared to the experience of these same children prior to HU therapy (i.e., 2.5 events per year before HU to 0.87 events per year on HU). Ferster et al. (13) also reported that after initiation of HU therapy, the 93 children in their study experienced significant decreases in both the number and duration of hospitalizations when compared to what had been observed in these same children during the 12 months prior to the initiation of HU therapy. Furthermore, an analysis of the subset of 22 children who had received HU for at least 5 years confirmed a significant difference in hospitalizations ($P = 0.0002$) as well as days in the hospital ($p < 0.01$). In addition to these clinical responses, both studies observed hematologic findings that were similar to those observed in the other adult and pediatric studies (i.e., increases in total Hb, % HbF and MCV and decreases

in circulating neutrophils, evidence of red cell destruction, etc). Therefore, just as in the adult studies, it remains unclear as to which of these various changes (or what combination of these changes) is responsible for the observed reduction in vaso-occlusive events.

With the increased understanding of the pathophysiology of SCD that we have gained over the past 10-15 years, it has become readily apparent that it is not simply the polymerization of hemoglobin S (HbS) and the formation of rigid, sickle erythrocytes (RBC) that leads to the impairment of blood flow and the resulting vaso-occlusion that is experienced by patients with SCD. We have learned, for example, that HbS-containing RBC (especially reticulocytes) are sticky and tend to adhere to one another, to the endothelium, and to the various proteins that comprise the subendothelial matrix. In addition, leukocytes, neutrophils, monocytes, inflammation, and blood clotting all appear to make important contributions to the process of vaso-occlusion. As emphasized above, HU can produce significant changes in many of these parameters. Furthermore, virtually all of the HU-induced changes tend to occur in the direction that would be of benefit in this clinical setting. As one example, Drs. Charache et al, in their extensive evaluation of the MSH (8), employed a multivariable analysis to provide convincing evidence of an independent association between lower neutrophil counts and lower crisis rates. By contrast, the increase in F-cell levels was associated with lower crisis rates, but only during the initial 3 months of HU therapy.

One final factor that is of critical importance to all of these studies relates to the issue of compliance. No matter how efficacious a therapy might be, it will only be effective if it is taken by the patient. In the original adult Phase I Study, for example, while those patients with the poorest HbF responses might have been refractory to the drug, it is important to emphasize that many of them were strongly suspected of noncompliance. Evidence of such noncompliance was suggested by the absence of HU in most of the random plasma samples from these “poor responders” (6). Similarly, in the HUG-KIDS Study, the extent of the increase in HbF level was inversely correlated with compliance with the treatment regimen as determined by pill counts (10). It is worth noting that Drs. Olivieri and Vischinsky conducted a study in children with SCD that was specifically designed to evaluate compliance with HU (14). By using the MEMS cap monitoring system, they found compliance to be remarkably high (96%) in their patient population. Perhaps because of parental supervision, it is quite possible that children with SCD may be substantially more compliant with the prescribed HU than are their adult counterparts. In any event, compliance is a vitally important factor in this setting, as we have all seen “HU-treated” patients with SCD whose hematological parameters (HbF, MCV, neutrophils, reticulocytes, etc) fail to change despite “taking” dosages of HU that often exceed 30 mg/kg/day.

In summary, it is readily apparent that when HU is administered to patients with SCD, it has a significant effect not just on the clinical expression of the disease, but also on a wide variety of laboratory parameters. Furthermore, in most cases these changes in

laboratory values tend to occur together (i.e., those patients who achieve the highest HbF levels also tend to have the most prominent increases in parameters such as total hemoglobin and MCV as well as the most striking declines in parameters such as total WBC, neutrophils, reticulocytes, and other markers of red cell destruction). Virtually all of these HU-induced changes in laboratory parameters occur in a direction that one would expect to be beneficial in the setting of SCD. It is therefore difficult to be certain whether one specific change (e.g., the increase in HbF) is responsible for the bulk of the observed clinical benefit and everything else is a secondary manifestation this primary effect, or alternatively whether the observed clinical benefit results from a combination of some or all of the various changes that occur in this clinical setting.

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