A Possible Mechanism of Heinz Body Hemolytic Anemia Induced by DQ-2511, a New Gastroprokinetic Drug, in Dogs

HIROSHI OHNO,* MAMORU NOMURA,* AND KAZUO WATANABE†

*Drug Safety Research Laboratory, Daiichi Pharmaceutical Company, Ltd., Tokyo R&D Center, 16-13, Kita-Kasai 1-Chome, Edogawa-ku, Tokyo 134, Japan; and †Laboratory of Chemical Pharmacology, Department of Drug Evaluation and Toxicology Sciences, Faculty of Pharmaceutical Sciences, Chiba University, 1-33, Yayoi-cho, Inage-ku, Chiba 263, Japan

Received December 26, 1995; accepted April 26, 1996


A previous study revealed that DQ-2511, a new gastroprokinetic drug, induced hemolytic anemia together with increased Heinz body formation, preceded by a marked decrease in erythrocyte reduced glutathione (GSH) content, after 2 weeks of dosing onward in dogs. In this study, the effect of DQ-2511 on erythrocytes in the early period of dosing, in comparison with that of β-acetylphenylhydrazine (APHZ), was investigated to confirm the difference between this drug and APHZ in the mechanism of increased Heinz body formation. DQ-2511 and APHZ were administered orally to beagle dogs for 1 week at dose levels of 600 and 4 mg/kg, respectively. Dogs receiving APHZ showed anemia after dosing for 7 days, together with an increase in methemoglobin and Heinz body formation after 3 days of dosing. In contrast, blood GSH, glutathione reductase, and γ-glutamylcysteine synthetase were only slightly decreased after dosing for 7 days. In dogs treated with DQ-2511, erythrocyte GSH began to decrease after 1 day of treatment and was about 25% of the control value after 7 days; however, no changes were seen in blood glutathione reductase, GSH peroxidase, or γ-glutamylcysteine synthetase level. Hepatic GSH was decreased slightly. In another experiment, SD rats were administered DQ-2511 and APHZ orally for 1 week at dose levels of 1600 and 15 mg/kg, respectively. Rats receiving DQ-2511 showed no anemia or any changes in erythrocyte GSH and Heinz body formation. In contrast, rats treated with APHZ showed a marked anemia and increases in Heinz body formation and erythrocyte GSH. These results demonstrate that DQ-2511 causes a marked decrease in GSH in dogs, resulting in Heinz body anemia, whereas APHZ induces Heinz body formation after a significant increase in methemoglobin, and suggest that impairment of the GSH redox cycle and synthetases of GSH are not involved in the decreased GSH after DQ-2511 treatment. This difference in effects on GSH content may indicate the existence of a species difference in the anemia induced by DQ-2511.

DQ-2511, 3-[2-(3,4-dimethoxyphenyl)ethylcarbamoyl-methyl]amino-N-methylbenzamide, was synthesized by Daiichi Pharmaceutical Company Ltd. (Tokyo, Japan) and originally developed as an antiulcer drug (Asano et al., 1990). On the basis of its enhancing effect on gastric emptying (Hatanaka et al., 1995), it is now under development as a therapeutic agent for epigastric distress in patients with chronic gastritis.

In subacute toxicity studies of this drug, dogs receiving 600 mg/kg DQ-2511 for 3 months developed anemia together with an increase in the number of Heinz body-containing cells. In contrast, dogs receiving 175 or 50 mg/kg did not show anemia, nor did rats receiving 100, 400, or 1600 mg/kg for 3 months. Thus, a species difference was seen in the induction of hemolytic anemia by this drug. No other evidence of toxicity was observed. In clinical studies of this drug, anemia and increased Heinz body formation were not seen in men administered 600 mg/day po.

In a previous study (Ohno et al., 1993), dogs receiving DQ-2511 at 600 mg/kg showed hemolytic anemia accompanied by an increase in the number of cells containing Heinz bodies, preceded by a marked decrease in erythrocyte reduced glutathione (GSH) concentration from 2 weeks of dosing onward; however, only a slight increase in the methemoglobin level was noted. In contrast, β-acetylphenylhydrazine (APHZ), a known inducer of hemolytic anemia with Heinz body formation and increased methemoglobin, caused hemolytic anemia accompanied by marked increases in both Heinz body-containing cells and methemoglobin concentration, but, inconsistent with the formation of Heinz bodies, no decrease in erythrocyte GSH concentration. These results suggested that a decrease in GSH in erythrocytes played an important role in the anemia and Heinz body formation induced by DQ-2511, but not in that by APHZ. In this previous study, however, hematological and biochemical parameters were determined only after 7 days of treatment; at this time, methemoglobin and Heinz body-containing cells were already markedly increased in dogs receiving APHZ, and GSH in erythrocytes was already markedly decreased in dogs receiving DQ-2511.

In the present study, therefore, we investigated the effects of DQ-2511 and APHZ on erythrocytes in the early period of dosing to confirm the difference in their mechanisms of...
increased Heinz body formation. We also investigated the effects of DQ-2511 on enzymes that play an important role in the redox cycle and synthesis of GSH to clarify the mechanism of its decrease in GSH in erythrocytes, in comparison with that of APHZ. Furthermore, to clarify the difference between rats and dogs in the anemia induced by this drug, its effects on Heinz body formation and GSH contents in erythrocytes in rats were also examined.

MATERIALS AND METHODS

Test Materials and Chemicals

DQ-2511 was synthesized by Daiichi Pharmaceutical Company, Ltd. The purity of the compound, Lot 2002, was 99.2%. APHZ was purchased from Tokyo Kasei Kogyo Company, Ltd. (Tokyo, Japan). 5,5'-Dithiobis(2-nitrobenzoic acid), reduced glutathione (GSH), oxidized glutathione (GSOG), adenosine 5'-triphosphate and glutathione reductase were obtained from Sigma Chemical Company (St. Louis, MO). 32P ATP (NEN Research Products) was purchased from Daiichi Pure Chemicals Company, Ltd. (Tokyo, Japan). All other chemicals used were of reagent grade.

Seven-Day Repeated Dose Study in Dogs

Animals. Twelve female pure-bred beagles were used in experiments 1 and 2. Animals were aged approximately 21 months when used in experiment 1 and approximately 28 months when used in experiment 2. They were acclimated to the animal room (temperature 23 ± 2°C, relative humidity 55 ± 15%, and a 12-hr light/dark cycle) prior to the start of the study. They were housed individually in suspended stainless-steel wire-mesh cages and were given approximately 300 g of dog chow (DS, Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water ad libitum.

Treatment. DQ-2511 or APHZ was administered orally to groups of four dogs once daily for 7 days at a dose of 600 or 4 mg/kg, respectively, in both experiments. DQ-2511 was administered as tablets prepared by Daiichi Pharmaceutical Company, Ltd., and APHZ in 1% gelatin capsules. The same number of dogs receiving placebo tablets served as controls. The animals were assigned different regimens in experiment 2 from those they were allotted in experiment 1.

Hematological and biochemical examinations. Blood samples were collected from all dogs before the commencement of administration and then before and 24 hours after dosing on Days 1, 3, and 7 in both experiments. Additional samples were also collected 2, 4, and 8 hr after dosing on Day 1 in experiment 1. EDTA-2K was added to all blood samples and the following items were measured: red blood cell count (RBC), hematocrit (Ht), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were measured with a fully automated hematology analyzer (E-5000, Toa Medical Electronics Co., Ltd., Kobe, Japan). Reticulocytes (Ret) were counted by Brecher's method. Methemoglobin was assayed according to the method of Van Kampen (Van Assendelft, 1970). Heinz bodies were measured according to Dacie and Lewis (1968). The erythrocyte GSH concentration was assayed according to Beutler et al. (1963). Activities of glutathione peroxidase and glutathione reductase were determined by the method of Beutler et al. (1977). γ-Glutamylcysteine synthetase and glutathione synthetase were assayed according to Beutler and Gehlin (1986). In experiment 2, liver was collected at the time of necropsy and the hepatic GSH content was determined according to Hisam and Hilt (1976).

Seven-Day Repeated Dose Study in Rats

Animals. Twenty-four male SD rats aged 10 weeks were used. The animals were acclimated to the animal room (temperature 23 ± 2°C, humidity 55 ± 15%, and a 12-hr light/dark cycle) for approximately 1 month prior to the start of the study. They were housed three animals per cage in suspended stainless-steel cages with wire-mesh floor and given pelleted rat diet (F-2, Funabashi Farm, Inc., Chiba, Japan) and tap water ad libitum.

Treatment. DQ-2511 was administered orally to groups of six rats once daily for 7 days at a dose of 400 or 1600 mg/kg. APHZ was given orally at a dose of 15 mg/kg for 7 days. For administration, DQ-2511 was suspended in 0.2% Tween 80/distilled water solution. APHZ was dissolved in distilled water. The same number of rats receiving 0.2% Tween 80 solution served as controls.

Hematological and biochemical examinations. Blood samples were collected from all rats 24 hours after the last dosing. EDTA-2K was added and the following items were measured by the above-mentioned methods: RBC, Ht, Hb, MCV, MCH, MCHC, Heinz bodies, and erythrocyte GSH.

Statistical Analysis

Means and standard errors were calculated for each group from all quantitative data. Values between treated groups and controls were compared by Dunnett's multiple comparison test. Statistical significance was set at p < 0.05 and p < 0.01.

RESULTS

Seven-Day Repeated Dose Study in Dogs

Dogs receiving 600 mg/kg of DQ-2511 showed no decrease in RBC (Fig. 1), Hb, or Ht and no increase in Heinz body-containing cells (Fig. 2) up to Day 7. These results are consistent with those of the previous study in which decreased red cell parameters and increased Heinz body formation were noted only after 14 days. No change was seen in methemoglobin (Fig. 3) during the treatment period. GSH content of erythrocytes decreased from 8 hr on Day 1 onward. This change progressed with time to reach about 25% of the control value on Day 7 (Fig. 4); however, no changes were seen in glutathione reductase (Fig. 5), glutathione peroxidase (Fig. 6), or γ-glutamylcysteine synthetase (Fig. 7) throughout the 7-day treatment period, nor was any decrease seen in glutathione synthetase on Day 3 (data not shown). Hepatic GSH content was decreased by only 25% compared with the control, even after dosing for 7 days (Fig. 8).

Dogs receiving 4 mg/kg of APHZ, on the other hand, showed significant decreases in RBC, Hb, and Ht from Day 3 onward. These decreases were most severe on Day 7, with values of about 60% of the corresponding pretreatment values. Together with these changes in red cell parameters, increases in Heinz body-containing cells, methemoglobin, and reticulocytes were noted from Day 3 onward. Unlike DQ-2511 treatment, however, administration of APHZ caused no change in the GSH content of erythrocytes on Day 3, which was the point at which increased Heinz body formation and anemia occurred, and only a slight decrease in GSH content on Day 7, i.e., after the anemia and increased Heinz body formation had developed. Consistent with the change in GSH, decreases in glutathione reductase and γ-glutamylcysteine synthetase and a tendency for increase in glutathione peroxidase were noted on day 7. No change was seen in hepatic GSH content.
FIG. 1. Red blood cell counts in dogs administered DQ-2511 for 7 days. ○, Control; ●, DQ-2511 600 mg/kg; ▲, β-acetylphenylhydrazine 4 mg/kg. Each value represents the mean ± SE (n = 4–8). **Significantly different from the control values (p < 0.01).

FIG. 2. Percentages and counts of Heinz body-containing cells in dogs administered DQ-2511 for 7 days. ○, Control; ●, DQ-2511 600 mg/kg; ▲, β-acetylphenylhydrazine 4 mg/kg. Each value represents the mean ± SE (n = 4). **Significantly different from the control values (p < 0.01).
FIG. 3. Percentages and concentrations of methemoglobin in dogs administered DQ-2511 for 7 days. O, Control; •, DQ-2511 600 mg/kg; ▲, β-acetylphenylhydrazine 4 mg/kg. Each value represents the mean ± SE (n = 4). *Significantly different from the control values (p < 0.05). **Significantly different from the control values (p < 0.01).

FIG. 4. Reduced glutathione (GSH) content of erythrocytes in dogs administered DQ-2511 for 7 days. O, Control; •, DQ-2511 600 mg/kg; ▲, β-acetylphenylhydrazine 4 mg/kg. Each value represents the mean ± SE (n = 4–8). *Significantly different from the control values (p < 0.05). **Significantly different from the control values (p < 0.01).
FIG. 5. Glutathione (GSSG) reductase activity in erythrocytes in dogs administered DQ-2511 for 7 days. O, Control; ●, DQ-2511 600 mg/kg; ▲, β-acetylphenylhydrazine 4 mg/kg. Each value represents the mean ± SE (n = 4). *Significantly different from the control values (p < 0.05).

FIG. 6. Glutathione (GSH) peroxidase activity in erythrocytes in dogs administered DQ-2511 for 7 days. O, Control; ●, DQ-2511 600 mg/kg; ▲, β-acetylphenylhydrazine 4 mg/kg. Each value represents the mean ± SE (n = 4). *Significantly different from the control values (p < 0.05).
OHNO, NOMURA, AND WATANABE

1.00
0.80
0.60
0.40
0.20
0.00
Predosing period 24 hr 24 hr 24 hr 24 hr
Time
FIG. 7. γ-Glutamylcysteine (γ-GC) synthetase activity in erythrocytes in dogs administered DQ-2511 for 7 days. O, Control; ●, DQ-2511 600 mg/kg; ▲, β-acetylphenylhydrazine 4 mg/kg. Each value represents the mean ± SE (n = 4). **Significantly different from the control values (p < 0.01).

globin and moves freely from one molecule of globin to another, and because heme-free globin is quite susceptible to denaturation. He also questioned whether methemoglobin is necessary as an intermediate compound in the oxidative destruction of hemoglobin, on the basis that methemoglobin does not always lead to hemolytic disease and that stabilizing methemoglobin by the addition of cyanide does not prevent the formation of Heinz bodies. It is thought that, when GSH content falls, the SH groups of the erythrocyte membrane tend to form S–S bonds with other SH groups from the membrane and with cytosolic protein, especially hemoglobin. This then leads to the formation of Heinz bodies due to S–S bond formation between denatured hemoglobin and the membranes of erythrocytes deficient in enzymes participating in the metabolic processes of the pentose phosphate pathway and the hexose monophosphate shunt (Bashan et al., 1982). Goto et al. (1993) indicated that erythrocyte GSH was indispensable for erythrocyte defense against oxidative damage and that sheep with GSH-deficient erythrocytes were more susceptible to oxidative agents. Thus, GSH may be involved in Heinz body formation.

In the present study, dogs receiving DQ-2511 showed a decrease in erythrocyte GSH content from 8 hr after dosing on Day 1. This decrease progressed with time to reach about 25% of control value after dosing for 7 days; however, no increase in methemoglobin was noted during the 7-day treatment period. Our previous study revealed that, from 2 weeks of dosing onward, DQ-2511 induced

Seven-Day Repeated Dose Study in Rats

DQ-2511 caused no change in RBC, Ht, Hb, erythrocyte GSH, or Heinz body-containing cell count even at 1600 mg/kg in rats (Fig. 9). Rats receiving APHZ, on the other hand, showed a marked decrease in red cell parameters together with a remarkable increase in Heinz body-containing cells after dosing for 7 days; however, GSH content in erythrocytes in the APHZ-treated rats increased to approximately twofold the control value.

DISCUSSION

It is generally considered that oxidation of hemoglobin results in the formation of methemoglobin and in the denaturation and precipitation of globin as Heinz bodies (Gordon-Smith, 1980; Nagel and Runney, 1973). There is, however, some controversy as to whether methemoglobin is an essential or important precursor for the formation of Heinz bodies. Rentsch (1968) reported that there is little correlation between the activities of methemoglobin and Heinz body formation, although the two are partly related. Beutler (1969) accepted the possibility that methemoglobin may be an intermediary in the oxidative destruction of hemoglobin because the heme group of methemoglobin is not bound tightly to

FIG. 8. Hepatic reduced glutathione (GSH) contents in dogs administered DQ-2511 for 7 days. O, Control; ●, DQ-2511 600 mg/kg. ▲, β-acetylphenylhydrazine 4 mg/kg. Each value represents the mean ± SE (n = 4). *Significantly different from the control values (p < 0.05)
hemolytic anemia together with increased Heinz body formation after a marked decrease in blood GSH content in dogs. It has been demonstrated that GSH plays an essential role in the maintenance of the physiological structure of erythrocytes, preventing the formation of interprotein or intraprotein disulfides within the membrane skeleton (Kosower and Kosower, 1983). Dapsone hydroxylamine induced the formation of disulfide-linked adducts between hemoglobin and the various skeletal proteins as well as between hemoglobin monomers, consistent with a decrease in erythrocyte GSH content (Grossman et al., 1992). It is also thought that depletion of erythrocyte GSH causes increased formation of Heinz bodies due to the S–S bond formation between denatured hemoglobin and the membranes of erythrocytes (Bashan et al., 1982). GSH depletion to about 20–30% of total glutathione level can impair the cellular defense (Reed, 1990). It is considered that erythrocytes are always exposed to endogenous oxidative stress. Therefore, these present and previous results suggest that a continuous marked decrease in erythrocyte GSH level is involved in the Heinz body formation in anemia induced by DQ-2511, but that methemoglobin takes little part.

The mechanism of Heinz body formation induced by APHZ and phenylhydrazine, typical compounds leading to Heinz body formation, is generally considered to be as follows: phenylhydrazine and oxyhemoglobin react to form methemoglobin, leading to irreversible hemichrome formation and resulting Heinz body formation (Nagel and Runney, 1973; Stern, 1985). As to the effect of APHZ on GSH concentration in erythrocytes, when dog erythrocytes were incubated with APHZ, erythrocyte GSH was decreased in the absence of glucose (Kurian and Iyer, 1977). In contrast, there was little or no decrease in GSH in the presence of glucose irrespective of increased formation of methemoglobin and Heinz bodies (Maeda et al., 1989; Ogawa et al., 1992). In our previous study in dogs receiving APHZ (Ohno et al., 1993), there was no decrease in erythrocyte GSH after dosing for 2 weeks, at which time anemia was most severe, although a slight decrease in erythrocyte GSH was noted after dosing for 1 week. In the present study, dogs treated with APHZ showed increases in methemoglobin and Heinz body-containing cells consistent with the appearance of anemia after dosing for 3 days; however, there was no evidence of a decrease in erythrocyte GSH level up to 3 days of dosing, whereas a slight decrease was seen after 7 days. These results suggest that methemoglobin formation plays a major role in Heinz body formation in the anemia induced by APHZ, whereas decreases in GSH are not directly involved.

GSH in erythrocytes is generally considered to participate in intracellular redox reactions to protect hemoglobin and some thiol-dependent enzymes or membrane proteins from oxidative damage. Its metabolism is controlled by uptake of its constituent amino acids, intracellular synthesis through catalysis by two enzymatic reactions, oxidation and reduction of GSH, and transport of degraded GSH to the outside of the cell. In detail, erythrocytes take up cysteine, glutamate, and glycine and synthesize GSH via γ-glutamylcysteine synthetase (γGC-S) and GSH synthetase (GSH-S). Both these
reactions require ATP. GSH is oxidized to GSSG by the action of GSH peroxidase (GPX) in the presence of peroxides such as hydrogen peroxide. Hydrogen peroxide is produced from superoxide anion, which is generated naturally or by chemical compounds, by superoxide dismutase. GSSG and mixed disulfides with protein, which are produced by oxidation of protein, are reduced to GSH through the action of glutathione reductase (GR). This reaction is coupled to the oxidation of NADPH. The supply of NADPH by the pentose phosphate pathway is dependent on glucose-6-phosphate dehydrogenase (6PD). GSSG, which affects various enzymes and membrane substances, is transported to the outside of erythrocytes.

The present study revealed that there were no changes in GPX, GR, yGC-S, or GSH-S in the DQ-2511-treated dogs. The previous study showed no decreases in G6PD and ATP, which play an important role in the maintenance of GSH level in erythrocytes. These results therefore suggest that the decrease in GSH level in erythrocytes after DQ-2511 treatment does not involve impairment of the GSH redox cycle or GSH synthesis.

On the other hand, APHZ did not decrease the activities of GR, GPX, yGC-S, or GSH-S up to dosing for 3 days, when GSH was not decreased. However, GR and y GC-S were decreased and GPX tended to increase after dosing for 7 days, when GSH was slightly decreased. The previous study revealed a slight increase in G6PD and no decrease in ATP after dosing for 7 days. From these results, the decreases in GR and yGC-S may contribute to the decreased GSH in APHZ-treated dogs; however, since no such changes were seen on dosing for up to 3 days, the changes observed might be due to an indirect effect caused by the marked increase in Heinz body formation induced by APHZ.

A subacute toxicity study of DQ-2511 in dogs revealed no histological changes in the liver, despite the development of anemia. In the present study, hepatic GSH content was only slightly decreased, whereas GSH content in erythrocytes decreased markedly. This difference in the effects of DQ-2511 may suggest a difference between the toxic effects of the compound on erythrocytes and liver.

In the subacute toxicity study of DQ-2511 in rats, no anemia occurred. Further, rats receiving DQ-2511 showed no sign of anemia or decrease in GSH in the present study. These results suggest that a species difference exists in the decrease in erythrocyte GSH in the anemia induced by DQ-2511. In contrast, APHZ-treated rats showed a marked increase in erythrocyte GSH content when anemia and a marked increase in Heinz body formation were noted. This result provides further evidence that a decrease in GSH is not directly involved in the anemia induced by APHZ.

In conclusion, the above results support the previous finding that DQ-2511 induces a marked decrease in the GSH content of erythrocytes prior to the formation of Heinz bodies and consequently causes hemolytic anemia without a clear increase in methemoglobin. In contrast, APHZ increases Heinz body formation consistent with increased methemoglobin without a decrease in GSH. Further, these results suggest that impairment of the GSH redox cycle and GSH synthesis is not involved in the DQ-2511-induced decrease in erythrocyte GSH content. These differences in effect on GSH content suggest a species difference in the anemia caused by DQ-2511 and a difference in toxic changes in erythrocytes and liver. The cause of this decrease in GSH content is still unclear, however, and further study is needed to clarify its mechanism.

REFERENCES


Grossman, S., Agar, N. S., Löffler, S., and Jollow, D. J. (1981). Carbonyl-mediated oxidation of sulfhydryl and mixed disulfides with protein, which are produced by oxidation of protein, are reduced to GSH through the action of glutathione reductase (GR). This reaction is coupled to the oxidation of NADPH. The supply of NADPH by the pentose phosphate pathway is dependent on glucose-6-phosphate dehydrogenase (G6PD). GSSG, which affects various enzymes and membrane substances, is transported to the outside of erythrocytes.

The present study revealed that there were no changes in GPX, GR, γGC-S, or GSH-S in the DQ-2511-treated dogs. The previous study showed no decreases in G6PD and ATP, which play an important role in the maintenance of GSH level in erythrocytes. These results therefore suggest that the decrease in GSH level in erythrocytes after DQ-2511 treatment does not involve impairment of the GSH redox cycle or GSH synthesis.

On the other hand, APHZ did not decrease the activities of GR, GPX, γGC-S, or GSH-S up to dosing for 3 days, when GSH was not decreased. However, GR and γ GC-S were decreased and GPX tended to increase after dosing for 7 days, when GSH was slightly decreased. The previous study revealed a slight increase in G6PD and no decrease in ATP after dosing for 7 days. From these results, the decreases in GR and γGC-S may contribute to the decreased GSH in APHZ-treated dogs; however, since no such changes were seen on dosing for up to 3 days, the changes observed might be due to an indirect effect caused by the marked increase in Heinz body formation induced by APHZ.

A subacute toxicity study of DQ-2511 in dogs revealed no histological changes in the liver, despite the development of anemia. In the present study, hepatic GSH content was only slightly decreased, whereas GSH content in erythrocytes decreased markedly. This difference in the effects of DQ-2511 may suggest a difference between the toxic effects of the compound on erythrocytes and liver.

In the subacute toxicity study of DQ-2511 in rats, no anemia occurred. Further, rats receiving DQ-2511 showed no sign of anemia or decrease in GSH in the present study. These results suggest that a species difference exists in the decrease in erythrocyte GSH in the anemia induced by DQ-2511. In contrast, APHZ-treated rats showed a marked increase in erythrocyte GSH content when anemia and a marked increase in Heinz body formation were noted. This result provides further evidence that a decrease in GSH is not directly involved in the anemia induced by APHZ.

In conclusion, the above results support the previous finding that DQ-2511 induces a marked decrease in the GSH content of erythrocytes prior to the formation of Heinz bodies and consequently causes hemolytic anemia without a clear increase in methemoglobin. In contrast, APHZ increases Heinz body formation consistent with increased methemoglobin without a decrease in GSH. Further, these results suggest that impairment of the GSH redox cycle and GSH synthesis is not involved in the DQ-2511-induced decrease in erythrocyte GSH content. These differences in effect on GSH content suggest a species difference in the anemia caused by DQ-2511 and a difference in toxic changes in erythrocytes and liver. The cause of this decrease in GSH content is still unclear, however, and further study is needed to clarify its mechanism.


