Effect of Onion Ingestion on Anti-oxidizing Agents in Dog Erythrocytes

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ABSTRACT. The effect of onion ingestion on red cell anti-oxidizing agents such as superoxide dismutase (SOD), catalase, glucose-6-phosphate dehydrogenase (G-6-PD), glutathione peroxidase (GPx), NADH-methemoglobin reductase and reduced glutathione (GSH) was studied in relation to Heinz body hemolytic anemia in dogs. To dogs was given the onion soup equivalent to 30 g of raw onion/kg body weight once a day for three days. A few days after the initiation of feeding, there was a transient decrease of SOD, catalase and G-6-PD activities to 60%, and GSH value to 70% of their predosing values with a sudden and marked Heinz body formation. These values then began to increase when Heinz bodies were observed in almost every red cells. On day 10, when red cells carrying Heinz bodies were almost cleared from blood circulation, catalase and G-6-PD reached peak values of nearly 250% of their predosing levels. Changes of GPx and NADH-methemoglobin reductase were not correlated with Heinz body counts. Methemoglobin concentration was significantly elevated and maintained in the level of 2–3% during the presence of Heinz bodies in the red cells.—**KEY WORDS:** anti-oxidizing agent, dog, Heinz body, methemoglobin, onion.

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It is well-known that onion ingestion causes Heinz body hemolytic anemia in dogs [7, 19, 28] and many other domestic animals [15, 17, 20, 27, 32]. However, the biochemical events that take place during hemolysis by onions are not yet fully studied.

Our previous studies using canine erythrocytes *in vitro* [26] have shown that the onion extract accelerated autoxidation of oxyhemoglobin to methemoglobin and its subsequent denaturation leading to the formation of Heinz bodies. Moreover, the generation of superoxide (O_2^-) and its role in erythrocyte membrane impairment were also demonstrated during the sequence of hemoglobin breakdown.

Red cells essentially possess various anti-oxidant substances including superoxide dismutase (SOD), catalase, glucose-6-phosphate dehydrogenase (G-6-PD), glutathione peroxidase (GPx) and methemoglobin reductase to protect cellular constituents from oxidative degradation. Only

when protective systems of antioxidants were hereditarily deficient or overwhelmed by oxidants, irreversible and destructive oxidation is liable to occur in red cells [5].

Drug-induced hemolysis is generally proposed to be a sequence of hemoglobin breakdown as well as inactivation of antioxidant systems within erythrocytes [23]. The present work was undertaken to study the effect of onions on these antioxidants *in vivo*. The correlation between Heinz body and methemoglobin or methemoglobinemia was also examined, since marked methemoglobin production was accompanied by Heinz body formation *in vitro* [26].

MATERIALS AND METHODS

Sliced onions (1 kg) were boiled in distilled water (1 l) for five minutes, and the soup collected by filtration through cotton cloth was concentrated to 200 ml under reduced pressure. One ml of this condensed onion

soup was equivalent to 5 g of raw onions.

Ten adult mongrel dogs (either sex) weighing 5–10 kg were subjected to clinical examinations before use. Each animal was kept in an individual kennel and fed on a mixture of dry dog food and bread twice a day. To five dogs was given the condensed onion soup equivalent to 30 g of raw onions/kg body weight once a day for 3 days. Others were used as controls.

Blood samples were collected by venipuncture into tubes containing acid-citrate-dextrose (ACD) solution. The hemoglobin content and packed cell volume (PCV) were determined by standard laboratory methods. Red cells containing Heinz bodies were counted by microscopic examination of whole blood stained with neutral red and brilliant green, and expressed as % of total red cells. GSH was measured by the method of Beutler *et al.* [1].

Methemoglobin was measured as described by Nakamura *et al.* [25] except that the amount of blood used was reduced to half.

Prior to enzyme assays, erythrocytes were freed of plasma, leucocytes and platelets by filtration through the 1:1 mixture of microcrystalline and α -cellulose, and followed by three washings with isotonic saline. The 1:20 hemolysate which consisted of 1 part washed erythrocytes, 1 part physiological saline and 18-part β -mercaptoethanol-EDTA solution was prepared by freezing and thawing the mixture.

For the determination of enzyme activities, Hitachi spectrophotometer model 220 equipped with a thermostatic cell holder was used, and all enzymes except SOD were assayed in a 2 ml reaction volume of reagent mixture with a silica cell at 37°C. SOD was measured by the method of Maral *et al.* [22] using O_2^- -dependent oxidation of nitroblue tetrazolium (NBT).

Catalase was assayed according to the method of Beutler [3] and the change of

optical density (OD) at 230 nm was measured.

G-6-PD activity was determined at 340 nm according to Ishida and Miwa [16], in which the total activity of G-6-PD and 6-phosphogluconate dehydrogenase (6-PGD) was measured first, and 6-PGD activity was next assayed in a reaction system without glucose-6-phosphate, the substrate for G-6-PD. G-6-PD activity was then obtained by subtracting 6-PGD activity from the total activity.

GPx was also assayed at 340 nm as described by Ishida and Miwa [16], and NADH-methemoglobin reductase was measured at 575 nm by the method of Beutler [3].

RESULTS

Severe hemolytic anemia was produced in all onion soup group showing hemoglobinemia and hemoglobinuria. One dog which died on day 5 showed severe jaundice in mucous membrane and liver without remarkable splenomegaly. PCV and hemoglobin concentration progressively decreased from the next day of onion feeding, and reached approximately 40% of their predosing value on day 5 (Fig. 1). Heinz bodies suddenly appeared in almost all red cells on day 2 and its high level continued during 4 days (Fig. 1). Methemoglobin concentration was elevated significantly in all onion group (Fig. 1). The dog which died on day 5 showed an exceptionally high value of methemoglobin, 15.3% on day 4 and 12.3% on day 5 just before death, while other dogs showed only a slight increase ranging 2–3% during the first 6 days. Methemoglobin then decreased toward the normal level, but the small amount of it was consistently detected during the Heinz body existence.

SOD activity of red cells decreased about 40% of the predosing value during the first

Fig. 1. Changes of PCV, Hb, Heinz body counts and methemoglobin in the dogs given the onion soup (●) and control dogs (○). Arrows indicate onion feeding. Each point represents mean $\pm$ SD. Significant differences from respective predosing mean values are indicated as * $P < 0.05$ and ** $P < 0.01$ (Student's t-test). Methemoglobin concentration in a dog which died on day 5 is indicated separately in a broken line.

Fig. 2. Change of SOD activity in dogs given the onion soup (●) and control dogs (○). Arrows indicate onion feeding. Each point represents mean $\pm$ SD. Significant differences from predosing mean value are indicated as * $P < 0.05$ and ** $P < 0.01$.

Fig. 3. Change of catalase activity in dogs given the onion soup (●) and control dogs (○). Arrows indicate onion feeding. Each point represents mean $\pm$ SD. Significant difference from predosing mean value is indicated as * $P < 0.05$.

Fig. 4. Change of G-6-PD activity in dogs given the onion soup (●) and control dogs (○). Arrows indicate onion feeding. Each point represents mean $\pm$ SD. Significant differences from predosing mean value are indicated as * $P < 0.05$.

Fig. 5. Change of GSH in dogs given the onion soup (●) and control dogs (○). Arrows indicate onion feeding. Each point represents mean $\pm$ SD. Significant difference from predosing mean value is indicated as * $P < 0.05$.

Fig. 6. Change of GPx activity in dogs given the onion soup (●) and control dogs (○). Arrows indicate onion feeding. Each point represents mean $\pm$ SD.

Fig. 7. Change of NADH methemoglobin reductase in dogs given onion soup (●) and control dogs (○). Arrows indicate onion feeding. Each point represents mean $\pm$ SD. Significant difference from predosing mean value is indicated as * $P < 0.05$.

24–48 h and markedly increased to 170% on day 5, followed by a slow and gradual decline toward the normal (Fig. 2).

Catalase and G-6-PD showed quite a similar pattern of changes (Figs. 3 and 4). Before the experiment, catalase activity was very low but individual difference was wide ($0.42\text{--}6.35 \times 10^3$ IU/g Hb). The activity decreased about 35% of the predosing value on day 3 and then increased slowly during the next 3 days followed by a marked

increase toward the peak value of 250%. G-6-PD activity also showed a transient decrease by 45% of the predosing value on day 3, and increased progressively during the next 7 days reaching the peak value of 240%. From day 10 to 14 both catalase and G-6-PD activities fell markedly, however, they were still elevated by 70–80% on day 14.

GSH concentration decreased slowly during the first 3 days, and the maximum decrease was 30% (Fig. 5). Then there was a moderate rise during the next several days followed by return to the predosing value on day 14.

GPx activity changed irregularly while NADH-methemoglobin reductase was moderately elevated during anemia (Figs. 6 and 7).

Four of six antioxidant substances examined, SOD, catalase, G-6-PD and GSH showed a significant depression when sudden and marked Heinz body production occurred. However, the decrease of them was transient and did not continue for more than a day or two, followed by progressive increase in spite of the high level presence of Heinz bodies in erythrocytes.

DISCUSSION

Effect of onion ingestion on substances involved in red cell anti-oxidizing systems was studied in relation to Heinz body and methemoglobin production. GSH value and SOD, catalase and G-6-PD activities significantly dropped when Heinz body counts reached the peak, but these substances progressively rose during clearance of erythrocytes carrying Heinz bodies from blood circulation. Methemoglobin was slightly elevated from normal level and detected continually during the presence of Heinz bodies in red cells.

Our previous *in vitro* work [26] has demonstrated that onions directly stimulate autoxidation of oxyhemoglobin to methemoglobin and superoxide (O_2^-), and methemoglobin thus produced is concurrently denatured into Heinz bodies while O_2^- and its metabolite H_2O_2 cause damage to the erythrocyte membrane.

The hemolytic compounds are either oxidizing agents or they lead to the formation of activated oxygens such as O_2^- , H_2O_2 , $\cdot OH$ and singlet oxygen (1O_2 : oxygen molecule in an excited reactive state) within red cells [5]. Small doses of hemolytic agents do not affect red cells so far as anti-oxidizing substances in red cells destroy activated oxygens or reduce methemoglobin back to hemoglobin. However, an application of hemolytic agents in larger doses would cause destructive oxidation of red cell constituents, since they may inhibit some anti-oxidizing enzymes and generate activated oxygens superior to reductive potential of red cells.

Brenner and Allison[4] suggested that inhibition of catalase might be important in the oxidative damage of erythrocytes induced by chemical substances, since compounds capable of producing Heinz bodies were also catalase inhibitors. Tudhope and Leece [31] reexamined the effect of various

compounds producing marked Heinz bodies and methemoglobin on anti-oxidizing enzymes in red cells. They found that catalase was inhibited by many compounds including ascorbic acid, menadione, sodium azide, primaquine, acetylphenyl hydrazine and potassium chlorate, whereas glutathione peroxidase and G-6-PD were inhibited by menadione only. It was also indicated that there was no close correlation between the degree of catalase inhibition and the Heinz body count or methemoglobin concentration.

In the present study, catalase activity in dog erythrocytes was $4.6 \times 10^3 \pm 2.5 \times 10^3$ IU/g Hb (mean $\pm$ SD, N=10) before onion feeding, and this was approximately one fortieth of that in human erythrocytes ($15.31 \times 10^4 \pm 2.39 \times 10^4$ IU/g Hb by Beutler [1]. Though catalase activity is very low in dog erythrocytes, its importance is evident from the fact that hemolysate freed of catalase prepared by sephadex DE-52 column is ready to undergo autoxidation of oxyhemoglobin to methemoglobin at room temperature (Fig. was not shown). Very low catalase activity in dog erythrocytes and its inhibition by onion feeding may provide one reason for frequent occurrence of onion poisoning in this species.

In contrast to catalase, values of GSH [21], SOD [14] and G-6-PD [8] in dog erythrocytes were comparable to those of humans [3, 14]. Onion feeding also caused a significant decrease of their values as well as it inhibited catalase during Heinz body production. Decrease of GSH value was probably correlated with inactivation of G-6-PD, since reproduction of GSH requires sufficient NADPH depending on G-6-PD activity [33].

During recovery from anemia, there was a progressive increase of SOD, catalase, G-6-PD and GSH. The pattern of changes was very similar to that of GPx and glutathione reductase in phenylhydrazine

poisoning in guinea-pigs by Suzuki and Kataoka [29]. Because values of these anti-oxidizing agents depend on the age of red cells [9, 10], their marked increase during and after anemia would be an evidence of regenerated young cells in the blood circulation.

GPx and NADH-methemoglobin reductase changed differently from other anti-oxidizing agents. GPx activity showed erratic change during anemia, and the cause is unknown at present. NADH-methemoglobin reductase was moderately elevated from the predosing level, which probably contributed to the repression of methemoglobin production, keeping a small quantity of 2–3%. This enzyme is generally known as a major agent of reducing methemoglobin back to oxyhemoglobin, and its hereditary deficiency in human red cells is associated with methemoglobinemia [30].

Methemoglobin had been believed to be an essential preliminary step in oxidative degradation of hemoglobin and also to be a precursor of Heinz bodies [12, 13, 18], but recent reports suggest that formation of methemoglobin and Heinz bodies are parallel processes and methemoglobin is not an essential precursor of Heinz bodies [2, 31]. From the results obtained, relationship between methemoglobin formation and Heinz body production is likely to agree with the latter view. Degrees of methemoglobin and Heinz body production vary widely with hemolytic drugs used, and there is no quantitative correlation between levels of these two pigments [24]. Onion is one of hemolytic agents which produce marked Heinz bodies and very small amount of methemoglobin resembling the effect of menadione on human erythrocytes. Slight methemoglobinemia is now one of clinical features of onion-caused hemolytic anemia in dogs. One should, however, be careful about a marked increase of methemoglobin although it is rare, as it often exacerbates

symptoms of anemia and results in death of animals.

REFERENCES

1. Beutler, E., Duron, O., and Kelly, B. M. 1963. Improved method for the determination of blood glutathione. *J. Lab. Clin. Med.* 61: 882–888.
2. Beutler, E. 1969. Drug-induced hemolytic anemia. *Pharmacological Reviews* 21: 73–103.
3. Beutler, E. 1975. *Red Blood Cell Metabolism*. 2nd edn., Grune and Stratton, New York.
4. Brenner, S., and Allison, A. C. 1953. Catalase inhibition: A possible mechanism for the production of Heinz bodies in erythrocytes. *Experientia* 10: 381–384.
5. Carrell, R. W., Winterbourn, C. C., and Rachmilewitz, E. A. 1975. Activated oxygen and hemolysis. *Br. J. Haematol.* 30: 259–264.
6. Desforges, J. F., Kalaw, E., and Gilchrist, P. 1960. Inhibition of glucose-6-phosphate dehydrogenase by hemolysis inducing drugs. *J. Lab. Clin. Med.* 55: 757–766.
7. Farkas, M. C., and Farkas, J. N. 1974. Hemolytic anemia due to ingestion of onion in a dog. *J.A.A.H.A.* 10: 65–66.
8. Giger, U., Harley, J. W., Yamaguchi, R. A., McNulty, P. K., Chiapella, A., and Beutler, E. 1985. Inherited phosphofructokinase deficiency in dogs with hyperventilation-induced hemolysis: Increased *in vitro* and *in vivo* alkaline fragility of erythrocytes. *Blood* 65: 345–351.
9. Glass, G. A., and Gershon, D. 1981. Enzymatic changes in rat erythrocytes with increasing cell and donor age: Loss of superoxide dismutase activity associated with increases in catalytically defective forms. *Biochem. Biophys. Res. Comm.* 103: 1245–1253.
10. Glass, G. A., and Gershon, D. 1984. Decreased enzymic protection and increased sensitivity to oxidative damage in erythrocytes as a function of cell and donor aging. *Biochem. J.* 218: 531–537.
11. Grimes, A. J. 1965. Symposium: Haemolytic anemias. *Proc. Royal Soc. Med.* 58: 19–22.
12. Harley, J. D., and Mauer, A. M. 1960. Studies on the formation of Heinz bodies. I. Methemoglobin production and oxyhemoglobin destruction. *Blood* 16: 1722–1735.
13. Harley, J. D., and Mauer, A. M. 1961. Studies on the formation of Heinz bodies. II. The nature and significance of Heinz bodies. *Blood* 17: 418–433.
14. Hobara, T., Kobayashi, H., Higashihara, E., Iwamoto, S., Hirota, H., Hashizume, A., and Sakai, T. 1981. Levels of superoxide dismutase activities of erythrocytes and several tissues in normal dogs. *Yamaguchi Igaku* 30: 311–315 (in

- Japanese).
15. Huchison, T.W.S. 1977. Onions as a cause of Heinz body anemia and death in cattle. *Can. Vet. J.* 18: 358-360.
 16. Ishida, Y., and Miwa, S. 1973. Measurement of red cell enzyme activities. *Jpn. J. Clin. Med.* 37: 806-810 (in Japanese).
 17. James, L. F., and Binns, W. 1966. Effects of feeding wild onions (*allium validum*) to bred ewes. *J. Am. Vet. Med. Assoc.* 149: 512-514.
 18. Jandl, J. H., Engle, L. K., and Allen, D. W. 1960. Oxidative hemolysis and precipitation of hemoglobin. I. Heinz body anemias as an acceleration of red cell ageing. *J. Clin. Invest.* 39: 1818-1836.
 19. Jeffrey, M. K. 1983. Onion toxicity in a dog. *Med. Vet. Practice* 64: 477-479.
 20. Koger, L. M. 1956. Onion poisoning in cattle. *J. Am. Vet. Med. Assoc.* 129: 75.
 21. Maede, Y., Kasai, N., and Taniguchi, N. 1982. Hereditary high concentration of glutathione in the canine erythrocytes associated with high accumulation of glutamate, glutamine and aspartate. *Blood* 59: 883-889.
 22. Maral, J., Pujet, K., and Michelson, A. M. 1977. Comparative study of superoxide dismutase, catalase and glutathione peroxidase levels in erythrocytes of different animals. *Biochem. Biophys. Res. Commun.* 77: 1525-1535.
 23. McMahon, S., and Stern, A. 1979. The interrelationship of superoxide dismutase and peroxidatic enzymes in the red cell. *Biochem. Biophys. Acta.* 566: 253-258.
 24. Miller, A., and Smith, H. C. 1970. The intracellular and membrane effects of oxidant agents on normal red cells. *Br. J. Haematol.* 19: 417-428.
 25. Nakamura, I., Nishida, N., Maruyama, H., Kudo, Y., and Kagami, M. 1980. Microdetermination of methemoglobin and the normal value. *St. Marianna Med. J.* 8: 146-152 (in Japanese).
 26. Ogawa, E., Akahori, F., and Kobayashi, K. 1985. *In vitro* studies on the breakdown of canine erythrocytes exposed to the onion extract. *Jpn. J. Vet. Sci.* 47: 719-729.
 27. Pierce, K. R., Joice, J. R., England, R. B., and Jones, L. P. 1972. Acute hemolytic anemia caused by wild onion poisoning in horses. *J. Am. Vet. Med. Assoc.* 160: 323-327.
 28. Spice, R. N. 1976. Hemolytic anemia associated with ingestion of onions in a dog. *Can. Vet. J.* 17: 181-183.
 29. Suzuki, M., and Kataoka, T. 1983. Effect of experimental anemia on red cell GSH and enzyme activities in guinea-pig and rabbit. *Comp. Biochem. Physiol.* 75B: 5-7.
 30. Takeshita, M., Tamura, M., Yubisui, T., and Yoneyama, Y. 1983. Exponential decay of cytochrome b₅ and cytochrome b reductase during senescence of erythrocytes: relation to the increased methemoglobin content. *J. Biochem.* 93: 931-934.
 31. Tudhope, G. R., and Leece, S. P. 1971. Red cell catalase and production of methemoglobin, Heinz bodies and changes of osmotic fragility due to drugs. *Acta Haemat.* 45: 290-302.
 32. Van Kampen, K. R., James, L. F., and Johnson, A. E. 1970. Hemolytic anemia in sheep fed with wild onion (*allium validum*). *J. Am. Vet. Med. Assoc.* 156: 328-332.
 33. Waller, H. D., and Benohr, H. C. 1974. Metabolic disorders in red blood cells. pp. 377-407. In: *Cellular and Molecular Biology of Erythrocytes* (Yoshikawa, H., and Repoport, S. M. eds.), University of Tokyo Press.

要 約

犬のタマネギ摂取による赤血球内抗酸化物質への影響：小川絵里・篠木忠・赤堀文昭・政岡俊夫（麻布大学獣医学部薬理学教室）——犬のタマネギ摂取による赤血球内抗酸化物質（SOD，カタラーゼ，G-6-PD，GSH，グルタチオンペルオキシダーゼ，NADH-メトヘモグロビンリダクターゼ）への影響を，Heinz 小体性溶血性貧血との関連において検討した。タマネギ煮汁を生タマネギ換算で30g/kg 1日1回連続3日間投与すると，初回投与後2，3日にHeinz小体の急激な増加にともなう，SOD，カタラーゼ，G-6-PDは投与前の約60％に，GSHは約70％に減少した。これらの抗酸化物質の低下は一過性で，Heinz小体出現率が100％近くを維持する間に徐々に上昇し，Heinz小体を含む赤血球が血流中からほとんど消失する10日目には投与前の約2倍に達した。グルタチオンペルオキシダーゼおよびNADH-メトヘモグロビンリダクターゼはHeinz小体増加と関連した変動を示さなかった。メトヘモグロビンはHeinz小体出現にともない有意に増加したが，2－3％の低レベルで推移し，小体の出現率とは無関係であった。