ウシ血清中および血漿中チオバルビツール酸反応物（TBARS）濃度の比較

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The Comparison of Thiobarbituric Acid Reactive Substances (TBARS) Concentrations in Plasma and Serum from Dairy Cattle

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ABSTRACT. The aim of this study was to compare thiobarbituric acid reactive substances (TBARS) concentrations in serum, plasma with heparin (heparin plasma), and plasma with ethylenediaminetetraacetic acid disodium salt (EDTA plasma) as anticoagulants from dairy cattle. Serum, heparin plasma, and EDTA plasma TBARS were not sufficiently strongly correlated to allow accurate prediction of one set of values from the other. Heparin plasma TBARS concentrations were found to be lower, and were affected by the duration of mixing during the assay process. The results suggest that it is necessary to differentiate TBARS concentrations between different sample types such as serum, heparin plasma, and EDTA plasma. For measurements of TBARS concentrations in cattle, EDTA plasma samples may be more suitable than the other samples.

KEY WORDS: dairy cattle, EDTA plasma, TBARS.

Oxidative stress is caused by an imbalance between oxygen radicals and the neutralizing capacity of antioxidants within living organisms [2, 12, 14]. Recently, it has been reported that oxidative stress is one of the factors that induces not only mastitis but also the other production diseases [2, 3, 5, 7, 11, 12, 14, 17]. Oxidative stress can be monitored using several biomarkers (antioxidants and pro-oxidants) [15]. Among these biomarkers, plasma conditions of thiobarbituric acid reactive substances (TBARS) are indicative of lipid peroxidation, and oxidative stress can lead to an increase in TBARS [6]. The measurement of TBARS concentrations in cattle blood is thus an important tool for evaluation of the health status of dairy cattle and therefore for the improvement of quality, efficiency and safety in milk production. Armstrong and Browne [1] reported that plasma TBARS are considered a good general indicator of oxidative stress. However, although TBARS indicate the level of oxidative stress they do not provide information on the quantity of the specific lipid peroxide [1]. The value of TBARS differs greatly depending on analytical conditions, for example, the presence or absence of anticoagulants and the nature of the anticoagulant can cause variation in measured TBARS concentrations [4, 8–10]. Our previous data (not published) showed that the serum TBARS concentrations in the cows which had mastitis were different from those of the plasma with ethylenediaminetetraacetic acid disodium salt (EDTA) TBARS concentrations, and those indicated that the components of the measured TBARS in the serum and in the plasma with EDTA were different. However, the concentrations of TBARS in serum or plasma from cows has rarely been compared. The objective of this study was to investigate variation in TBARS concentrations from serum and plasma with anticoagulants, and to evaluate the blood sample types that are suitable for the measurement of TBARS concentrations in the cattle.

A total of 126 blood samples were randomly obtained in 42 collections from 12 individual cows. Blood samples were collected from the jugular vein using 3 kinds of plastic vacuum tubes: 1) tubes with EDTA (Venoject II VP-NA070K, Terumo Co., Ltd.) for plasma, 2) tubes with heparin (Venoject II VP-H100K, Terumo Co., Ltd.) for plasma, and 3) tubes without any inhibitors of blood clotting (Venoject II VP-P100, Terumo Co., Ltd.) for serum, at each collecting time. The tubes with EDTA and heparin were immediately placed on ice and then centrifuged at 3,000 rpm for 30 min at 5°C. The plasma was stored at –30°C until analysis. The tubes for serum were allowed to clot at room temperature for more than 40 min before centrifugation at 3,000 rpm for 30 min at 5°C. The serum was then stored at –30°C until analysis.

TBARS concentrations were measured fluorometrically using a TBARS assay kit (Oxitex TBARS Assay Kit, Zepto Metrix Co., New York) according to the manufacturer’s instructions. This represented an adaptation of the Yagi method [18, 19] for the samples with plasma EDTA, the method of LeFevre et al. [10] for the serum samples, and LeFevre et al. [10] and Gidez et al. [4] for the plasma with heparin. In the process of following the manufacturer’s protocol [4, 10] for plasma samples with heparin, when the pellet of precipitated oxidized low-density lipoprotein and/or oxidized very-low-density lipoprotein (LDL/VLDL) was resuspended in saline, the pellet with heparin was difficult to dissolve. We therefore carried out the mixing to dissolve the LDL/VLDL pellet with saline for 2 different time durations, namely (1) 3 min (3 min heparin), and (2) 7 min (7 min heparin) at room temperature. After 3 min mixing and 7 min mixing, the pellet remained insoluble in approxi-
mately 50% of samples and 80% of samples, respectively. If the insoluble LDL/VLDL pellets remained after these mixing durations, we left them for a further period and proceeded to the next step of protocol. In preliminary assays, pellets which remained after 7 min mixing did not dissolve even after more than 20 min mixing time. Since prolonged mixing and heating may causes TBARS degeneration, we used only the saline solvent portion of the sample and disregarded the insoluble component.

Pearson’s correlation coefficient was calculated using the CORR procedure of SAS [16] among TBARS concentrations with serum (serum TBARS), 3 min heparin (3 min heparin TBARS), 7 min heparin (7 min heparin TBARS), and EDTA (EDTA TBARS). When significant correlation coefficients were observed, regression analysis was conducted using the simple linear regression model procedure of SAS, PROC REG [16]. The effect of the different methods of treating the blood samples on the TBARS concentrations was evaluated by analysis of variance using the MIXED procedure of SAS [16].

The comparisons of the EDTA, 3 min heparin, 7 min heparin, and serum TBARS is shown in Fig. 1. The TBARS concentrations in the four different kinds of blood samples decreased in the order EDTA > serum > 7 min heparin > 3 min heparin (P<0.01). TBARS concentrations from EDTA samples were 1.4- to 14-fold higher than those of serum and approximately 2- to more than 20-fold higher than those of plasma samples containing heparin. The minimum and maximum values of the TBARS measurement concentrations in the blood samples used in this study ranged 0.50 to 1.39 nmol/ml with EDTA, 0.06 to 0.49 nmol/ml with serum, 0.00 to 0.27 nmol/ml with 3 min heparin, and 0.07 to 0.28 nmol/ml with 7 min heparin. Furthermore, there was no correlation between serum TBARS concentrations and EDTA TBARS (r=0.19) (Fig. 1a). The EDTA TBARS concentrations were not correlated with 3 min heparin TBARS (r=-0.09) (Fig. 1b) but were loosely correlated with 7 min heparin plasma samples (r=0.39; P<0.025) (Fig. 1c). The serum samples TBARS concentrations were approximately 2-fold higher than those of the 3 min and 7 min heparin samples. The serum TBARS correlated poorly with those of 3 min heparin (r=0.65; P<0.01) (Fig. 1d) and 7 min heparin samples (r=0.48; P<0.01) (Fig. 1e). However, the significant correlations between the 3 min heparin TBARS and the 7 min heparin TBARS was not observed (r=0.1907) (Fig. 1f).

Armstrong and Browne [1] and Kojima et al. [9] suggested that TBARS concentration cannot indicate the quantity of a specific lipid peroxide. Furthermore, unlike in edible oil and liver homogenate, the TBARS concentration in plasma does not specify a characteristic profile of pigment formation [9]. Also, previous work has shown that the chromogenic reaction level with thiobarbituric acid is affected by pH and the presence of EDTA [8, 9]. The results of the current study indicated that EDTA TBARS was markedly higher than that of the serum and plasma samples with heparin. In addition, the EDTA TBARS concentration was not correlated with serum TBARS concentration. The EDTA TBARS concentration and the heparin plasma, or serum TBARS concentrations are likely to represent different components. Although 7 min heparin TBARS concentration was loosely correlated with EDTA TBARS concentration, the relationship was not sufficiently strong to allow estimation of EDTA TBARS concentration from the heparin TBARS values. The relationship between serum TBARS concentration and heparin TBARS concentration is better than the relationship between the EDTA TBARS concentration and the heparin TBARS concentration. Even so, the correlation coefficients were not high enough to allow accurate estimation of serum TBARS concentrations from the heparin TBARS concentrations. In addition, it is conceivable that the correlation between 7 min heparin TBARS concentration and EDTA TBARS concentration was an artifact, since the range of concentrations was lower, probably reflecting the excessively long mixing times in the 7 min heparin samples. The connection between 3 min heparin and 7 min heparin suggests that the longer mixing time to resolve the precipitation does not always improve the result. Unnecessary mixing time at room temperature reduces the concentrations of TBARS in some of the heparin plasma samples. These data variations might be caused by variable time to clot for serum separation, room temperature during that period, and mixing time to resolve LDL/VLDL precipitation in the assay process for heparin plasma.

Recent studies have shown that the red pigment which is detected as TBARS includes alkenal and alkadienal, as well as the main component of the condensate with thiobarbituric acid and malonaldehyde, and that formation of the red pigment is enhanced by tert-butyl hydroperoxide (tert-BuOOH) and ferric ion (Fe3+) without EDTA [8, 9]. In the presence of EDTA, these chromogenic reactions which are derived by alkenal/alkadienal, tert-BuOOH, and Fe3+, are completely inhibited by EDTA except in the case of malonaldehyde [8, 9]. Although the origin of TBARS in plasma has not yet been clarified [9], our results imply that the TBARS assay in the plasma with EDTA might be more useful in providing a relatively wide range of analytical values and a simpler analytical process. It avoids the problems of the LDL/VLDL pellet which precipitates from serum and from the plasma with heparin samples. Furthermore, the TBARS assay from the plasma heparin was susceptible to variations in the analytical process and was not particularly instructive. Niki [13] stated that if the samples were confined, such as with plasma, and if the values were measured under as constant analytical conditions as possible, the obtained values for TBARS would be as worth discussing as the relative values. Therefore EDTA plasma samples may be more suitable than the other samples for measurements of TBARS concentrations in cattle, because the values from EDTA plasma seem most robust with respect to the effects of assay preparation and processing.

From these results, we suggest that serum TBARS concentration, heparin-containing plasma TBARS concentration, and the EDTA-containing plasma TBARS
Concentrations are very different, and it is impossible to predict one set of values from another. We recommend that the EDTA plasma be used for determining the TBARS concentrations in cattle blood.

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REFERENCES