Mac Vet Rev 2013; 36 (2): 85–89

Available online at
www.macvetrev.mk

Original Scientific Article

UDC: 636.2.09:616-008.9

BILIARY CLEARANCE OF BROMOSULFOPHTHALEIN IN HEALTHY AND KETOTIC HOLSTEIN COWS

Kirovski Danijela[1], Sladojević Željko[2], Šamanc Horea[3]

[1]*Department of Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Belgrade, 11000 Belgrade, Serbia*

[2]*Veterinary Station „Veterina system Sladojević“, 78400 Gradiška Bosna and Herzegovina, Republic of Srpska*

[3]*Department of Farm Animal Disease, Faculty of Veterinary Medicine, University of Belgrade, 11000 Belgrade, Serbia*

Received 30 June 2013; Received in revised form 14 July 2013; Accepted 20 August 2013

ABSTRACT

Ketosis is a metabolic disorder closely associated with liver lipidosis. Numerous tests have been developed to detect hepatic dysfunction in dairy cows. Bromosulfophthalein (BSP) clearance is established as a sensitive index of hepatic function. The objective of this study was to examine the difference of biliary excretion of BSP between ketotic and healthy Holstein cows and to correlate this excretion with other indicators of liver dysfunction. Twenty puerperal Holstein cows divided in two groups (10 cows each) were involved in the study. The first group included healthy and the second group ketotic cows. Blood samples were taken 10 days after parturition. Concentrations of total protein, albumin, total bilirubin, Ca, P, total lipids, urea and glucose were determined. Immediately after blood sampling, BSP test was performed. Blood samples were taken 5 and 45 minutes after injection, and the percentage of retained pigment in the sample obtained at minute 45 was calculated. Blood albumin and glucose concentrations were significantly higher in healthy then ketotic cows. Total bilirubin concentration was significantly higher in ketotic than healthy cows. BSP excretion was significantly higher in ketotic compared to healthy cows. There was a significant positive correlation between BSP values and total bilirubin concentrartions in both healthy and ketotic cows and a significant negative correlation between BSP values and glucose concentrartions in both healthy and ketotic cows. In conclusion, biliary clearance of BSP may be used as a reliable method for the detection of hepatic dysfunction associated with clinical symptoms of ketosis in dairy cows.

Key words: Holstein cows, BSP test, ketosis

INTRODUCTION

Dairy cows are highly susceptible to developing ketosis. Ketosis is a metabolic disorder caracterized by alterations in the metabolism of carbohydrates and lipids. High milk productive, stressed, and cows with hormonal disbalance are predisposed to this disorder which is usually combined with hypoglycemia, hyperketonemia and ketonuria (10).

During early lactation the mammary gland has a priority in nutrient supply, even in the case of clinical ketosis (7, 19). In hypoglicemic ketotic cows the mammary gland uses glucose in the same quantity as the mammary gland in cows with normal glycemia. It indicates that in most critical phases of lactation, when the metabolic processes are on the edge of developing into the ketotic state, the regulatory role of hormones and gluconeogenetic capacity of the liver are of crucial importance (15). The liver capacity to synthesize sufficient quantities of glucose for metabolic needs is most important during pregnancy and lactation. During this period the gluconeogenetic capacity of the liver is disrupted mainly by fatty liver. Fatty liver is a consequence

Corresponding author: Prof. Kirovski Danijela, DVM, PhD
E-mail address: dani@vet.bg.ac.rs
Present address: Faculty of Veterinary Medicine, University of Belgrade, Studentski Trg 1, 11000 Belgrade, Serbia
tel: +381 11 3207 400 fax: +381 11 2638 912

Competing Interests: The authors have declared that no competing interests exist.

of enhanced and uncontrolled lipid mobilization from body reserves (4). Earlier studies indicate that in ketotic cows fatty liver is very frequent and is crucial for the outcome of the disease. Some authors indicate that cows with severe hepatosteatosis develop ketosis more often than cows with mild fatty liver. Nevertheless it may be supposed that ketosis is a metabolic disorder closely associated with liver lipidosis and that those two diseases are the two most important metabolic disorders during early lactation (6). This is the reason why researchers are still focused on pathomorphological changes in the liver and metabolic tests that can serve as valid indicators of its functional state. Numerous tests have been developed to detect hepatic dysfunction in dairy cows (12). Among others, bromosulfophthalein (BSP) clearance is established as a sensitive index of hepatic function (5, 9).

Bromsulphalein (BSP) test is rarely used in veterinary medicine, especially for monitorinig the excretory capacity of the liver in ketotic cows. For normal pigment excretion, liver blood circulation, hepatocyte function and passage of bile canalicules should be preserved (3, 13). If the liver is healthy, only traces of the total pigment remained in the blood serum 45 minutes after BSP injection. According to Heidrich et al. (8) BSP test is acceptable in cattle to detect liver demage, although greater deviations in pigment retention can be expected in severe liver damages, only.

The objective of this study is to examine the difference of BSP excretion between ketotic and healthy Holstein cows and to correlate these variables to some other indicators of liver dysfunction.

MATERIALS AND METHODS

Twenty puerperal (10 days after calving) Holstein cows were involved in the study. These were divided in two groups (n = 10). The first group of cows included healthy animals and the second group included cows with clinical symptoms of ketosis. The cows were defined as clinically ketotic if they had signs of inappetence, ruminal atony, depression and their urinary ketone body levels were higher than 17.2 mmol/L. Urine samples were qualitatively determined for ketone bodies using a Rothera test. Rothera's test detects acetone and aceto-acetate but not betahydroxybutyric acid (10). The intensity of the ring color was classified into N (negative), +1, + 2 and +3 based on the color reaction of the sodium nitroprusside (SNP) test with a known amount of acetone in aqueous solution at 0 mmol (N), 1 to 3.4 mmol (+1), 3.5 to 17.2 mmol (+2), more than 17.2 mmol (+3), respectively (13). Cases with secondary ketosis (i.e. ketosis that appeared to be secondary to other conditions) were not included in this study.

Blood samples from all cows were taken from the jugular vein just before BSP testing. Separated blood sera were stored at -20°C until use. Concentrations of total protein, albumin, total bilirubin, Ca, P, total lipids, urea and glucose were measured by photometric method using the biochemical analyzer (VetScreen, Biochemical Systems, Italy).

Commercial bromosulphophtalein (Phenoltetra-bromphtalein-dinatriumsulphonat, Darmstadt; 5 % solution) was used for the BSP test. Five mg of BSP per kg of body weight was slowly injected into the jugular vein. One and forty five minutes later blood samples from the contralateral jugular vein were taken. The concentration of BSP in the serum was determined spectrophotometrically. Percent of BSP retention in blood sera 45 minutes after injection was calculated as follows: BSP (%) = A45/A5 x 100 (A45-absorbance measured at wavelength 570 nm in blood samples obtained 45 minutes after injection; A5- absorbance measured at wavelength 570 nm in blood samples obtained 1 minute after injection)

RESULTS

Results for concentrations of main biochemical indicators of hepatic function in healthy and ketotic cows, as well as BSP retention are presented in Table 1.

Table 1. Concentrationas of main biochemical parameters (X ± SD) and BSP excretion (%) in healthy and ketotic cows

Parameter	Healthy cows	Ketotic cows	Signif. of diff. between groups
Total protein (g/L)	75,83 ± 4,99	73,44 ± 7,95	n.s
Albumin (g/L)	37,50 ± 4,15	31,39 ± 6,09	$p < 0,05$
Total bilirubin (mmol/L)	7,21 ± 3,64	18,01 ± 8,69	$p < 0,01$
Ca (mmol/L)	2,18 ± 0,20	2,19 ± 0,23	n.s
P (mmol/L)	1,61 ± 0,20	1,58 ± 0,30	n.s
Total lipids (mmol/L)	4,15 ± 0,78	3,67 ± 0,90	n.s
Urea (mmol/L)	3,84 ± 0,63	3,35 ± 1,07	n.s
Glucose (mmol/L)	2,57 ± 0,33	1,79 ± 0,40	$p < 0,001$
BSP (%)	4,20 ± 2,40	24,90 ± 11,10	$p < 0,001$

Blood albumin and glucose concentrations were significantly higher in healthy compared to ketotic cows ($p < 0.05$ and $p < 0.001$, respectively) while total bilirubin concentration was significantly higher in ketotic compared to healthy cows ($p < 0.01$). In healthy cows BSP was cleared rapidly from the circulation. When 45 minutes expired, only traces of pigment were present in the sera of healthy cows, while 24.90 ± 11.10 % of the pigment was retained in the blood of ketotic cows. As shown in Table 1, BSP excretion was significantly higher in ketotic compared to healthy cows ($p < 0.001$).

Corelation coefficients between BSP excretion values and main biochemical indicators of hepatic functions of healty and ketotic cows are presented in Table 2.

Table 2. Correlation bettwen BSP excretion (%) and main biochemical parameters in healthy and ketotic cows

Healthy cows	Coeficinet of correlation	Significance
Total protein	-0.130	n.s
Albumin	-0.250	n.s
Total bilirubin	0.760	$p < 0.01$
Urea	-0.500	n.s
Glucose	-0.816	$p < 0.01$
Ketotic cows		
Total protein	-0.400	n.s.
Albumin	-0.580	n.s
Total bilirubin	0.800	$p < 0.01$
Urea	-0.220	n.s.
Glucose	-0.740	$p < 0.01$

There was a significant positive correlation between BSP values and total bilirubin concentrartions in both healthy ($r = 0.760$; $p < 0.01$) and ketotic cows ($r = 0.800$; $p < 0.01$) and a significant negative correlation between BSP values and glucose concentrations in both healthy ($r = -0.816$; $p < 0.01$) and ketotic cows ($r = -0.740$; $p < 0.01$).

DISCUSSION

Our results for biochemical parametars that are valuable biomarkers of liver function in dairy cows (albumin, total bilirubin and glucose), indicate that synthetic processes in liver are impaired in ketotic cows. Namely, glucose concentration which is good indicator of gluconeogenetic capacity of liver (11) is significantly lower in ketotic cows. Albumin is synthetasied in liver (2) and therefore its concentration was significantly lower in ketotic compared to healthy cows. Total bilirubin concentration is wellknown indicator of fatty liver in dairy cows (1). Total bilirubin concentration was significantly higher in ketotic than healthy cows indicating liver dysfunction in diseased cows. Percent of BSP retaained in blood serum 45 minutes after application of pigment was significantly higher in ketotic cows indicating on slower uptake, conjugation and/or excretion of pigment throught biliary canalicus in ketotic cows. Namely, several investigators (9) have noted a time lag between the uptake of BSP by the liver and its eventual secretion into the bile. They suggested that clearance of the pigment by the liver involves three independent processes: uptake, conjugation and secretion. As known, BSP is mainly taken up by the liver through the organic anion transporting polypeptide families, conjugated with reduced glutathione (GSH) within the hepatocytes by the glutathione-S-transferase (GST), and subsequently excreted into bile by the multidrug resistance associated protein 2 (16), similarly to bilirubin. Impaired clearance of BSP could result from interference with any one or any combination of these processes (14). Our results that indicate on significant positive correlation between BSP retention and total bilirubin concentrations both in ketotic and healthy cows are in accordance with other authors (17). Some authors indicated that elevation of BSP metabolites may be apparent long before any rise in serum bilirubin or aspartate aminotransferase became evident (18). This preferential appearance could be entirely a concentration effect. On the other hand, it is suggested that secretion at the cellular level may involve metabolic processes, some of which are specifically inhibited by metabolic products of damaged liver, and this inhibition produces a "metabolic" obstruction. Due to strong positive correlation between BSP % and bilirubinemia, it has been suggested that measurement of BSP retention where hyperbilirubinemia exists does not give futher infomration about the liver function. Anyway, the sensitivity of the BSP test may depend on administration of large amounts of the pigment, sufficient to load the various systems involved in its metabolism. This process may be helpful in the detection of slight hepatic functional defects which are not reflected by changes in ie. serum bilirubin or aspartate aminotransferase levels. Since BSP is an exogenous substance, alteration of serum levels by its endogenous production, such as occurs with bilirubin, is not a complicating factor. In addition, BSP and bilirubin may reflect different functions of the liver: mercaptide synthesis and secretion by BSP and glucuronide and sulfate formation by bilirubin. The BSP test becomes in part a measure of a specific biochemical reaction in the liver. Under certain circumstances this reaction may be specifically inhibited while other liver functions proceed. Additionally, there is strong negative correlation between glucose concentrations and BSP excretion indicating that there is some interaction in gluconeogenetic pathways and BSP uptake, conjugation and/or excretion in dairy cows.

CONCLUSION

Based on the obtained results it may be concluded that biliary clearance of bromosulfophthalein may be used as a reliable method for the detection of hepatic dysfunction associated with clinical symptoms of ketosis in dairy cows. Additionally, total bilirubin and glucose concentrations may be good indicators of impaired liver function in puerperal cows.

ACKNOWLEDGEMENTS

This work was supported by Ministry of Science and Technology, Republic of Serbia, Project Grant No 46002.

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Please cite this article as: Kirovski Danijela, Sladojević Željko, Šamanc Horea. Biliary clearance of bromosulfophthalein in healthy and ketotic holstein cows. Mac Vet Rev 2013; 36 (2): 85-89.