Determination of Some Enzymes in the Urine of Bovine Animals With Renal Damage

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Summary

The authors evaluate the correlation between the alteration of urinary enzymatic activity in cows affected by kidney damage found in post-mortem and histological examination.

Introduction

Our previous research, carried out on the urine of healthy cattle, monitored the urinary activity of N-acetyl-glucosaminidase (NAG), Gamma-glutamyl-transferase (Gamma-GT), Lactate dedyrogenase (LDH), Alpha-hydroxybutyric dehydrogenase (Alpha HBDH) and Alkaline phosphatase (AP).(5)

The purpose of this study is to evaluate the correlation between the alteration of urinary enzymatic activity and the kidney damage found in post-mortem and histological examination.

Methods

The objects of our studies have been 55 healthy female Italian Fisona calves between 4 and 7 years old, destined to slaughterhouses. Before slaughtering we subjected all the animals to haematological examination with tube test without anticoagulant. The blood samples were centrifuged at 3000 rpm for ten minutes and the obtained serums were kept at +4°C. Within 5 hours from the collection of blood samples, we determined serum levels of creatinine, urea, total protein, LDH, alpha HBDH, Gamma GT and AP. (Table 1)

After slaughtering, we took urine samples that were kept in sterilized containers at +4°C until the moment of testing.

On urine samples we determined complete physical and clinical examination, the values of creatinine and microprotein and the enzymatic activity of NAG, LDH, alpha HBDH, AP and Gamma GT. (Table 1)

The enzymatic activity and microproteinuria results determined on single urine samples, were correlated with the urinary creatinine values to cancel out the possible physiological variations correlated with the "volume factor" (1,2,5).

After slaughtering we took both kidneys from each subject and post-mortem macroscopic and microscopic examinations were made which allowed us to identify 34 subjects with at least one kidney with lesions.

During the macroscopic examination of each kidney, we observed the external and cutting surface. In this way we were able to set the organs into two categories:

- without important macroscopic lesions (WML)
- with roundish grey-white focus measuring from a fraction of a millimeter to half a centimeter and continuing along the cortex.

These foci, typical of chronic interstitial nephritis (NIC), were marked with FOC+, FOC++, FOC+++, on the basis of their range and diffusion.

Moreover we found:
- one case of renal amyloidosis
- one kidney with chronic interstitial nephritis
- one lobular acute infarction
- one lobular old infarction like a connectival scar
- one case of severe bilateral pan-nephritis with infarctions detected in one animal with infectious pleuro-pneumonia.

To make histological examinations, we took 2 samples from every kidney:

- kidneys WML, random
- pathological kidneys, one from the lesions and one far from them.

The samples, kept in 10% formalin, set in paraffin and cut 5 micron thick, were stained with haematoxylon-eosin. The sample of kidney with amyloidosis was stained with Congo Red with positive result.

Results

The serum values of urea, creatinine, total protein and enzymatic activities, calculated on the 34 examined subjects were normal for the cow (7,9). At the end of the post-mortem macroscopic and microscopic examination of the kidneys we divided them in 4 classes with different seriousness (Tables 2,3,4,5). The same tables show the values of urinary enzymatic activities and microproteinuria correlated with creatininuria on the subject of 1, 2, 3, and 4 classes.

Table 6 shows the values of urinary enzymatic activities and microproteinuria correlated with creatininuria on the subjects with severe renal damage: amyloidosis, chronic interstitial nephritis, lobular acute infarction, lobular old infarction and severe pannephritis with infarctions.

Table 1.

UREAS	Enzymatic colorimetric method modified Berthelot's reaction
CREATININE S/U	Colorimetric Kinetic method with alkaline picrate
TOTAL PROTEIN	Biuret method
MICROPROTEINURIA	Blue Coomassie method
ALKALINE PHOSPHATASE S/U (AP)	p-Nitrophenylphophate kinetic method
GAMMA-GLUTAMYL-TRANSFERASE S/U (Gamma - GT)	Colorimetric kinetic method
LACTATE DEHYDROGENASE S/U (LDH)	Optimized standard method conforming to DGKCH
alpha HYDROXYBUTYRIC DEHYDROGENASE S/U (alpha-HBDH)	Optimized standard method conforming to DGKCH
N-ACETYL-β-GLUCOSAMINIDASE U. (NAG)	Colorimetric method

Table 2. Values of urinary enzymatic activities and microproteinuria in the first class.
- Macroscopic report: both kidneys WML only one FOC+
- Histologic report: small interstitial focus of lymphoplasma cells (NIC+)

	$\bar{X}$	1	2	3	4	5
NAG/C	0.20	0.15	0.30	0.23	0.21	0.11
AP/C	0.92	N.D	0.20	0.26	0.91	2.31
γGT/C	1.02	0.43	0.75	0.86	0.48	2.61
LDH/C	3.50	2.04	7.30	0.83	1.68	5.68
αHBDH/C	1.95	0.91	4.70	0.62	0.70	2.84
Microp. /C	0.15	0.12	0.08	0.37	0.04	0.17
C	11.31	18.56	5.30	23.86	7.07	1.76

ENZYMES: U/L; MICRPROTEINURIA mg/dl;
CREATININURIA (C): mmol/L
N.D. = Not detectable

Table 3. Values of urinary enzymatic ativities and microproteinuria in the second class.
- Macroscopic report: both kidneys WML or one FOC+ or both FOC+
- Microscopic report: both kidneys small interstitial focus of lymphoplasma cells (NIC+)

	$\bar{X}$	1	2	3	4	5	6	7	8	9	10	11	12
NAG/C	0.28	0.08	0.26	0.17	0.16	0.68	0.37	0.05	0.30	0.43	0.26	0.28	0.32
AP/C	0.63	0.23	0.58	0.05	0.28	N.D.	1.69	0.46	1.48	N.D.	0.42	0.26	N.D.
γGT/C	0.64	0.68	0.12	0.34	0.63	2.04	1.31	0.17	0.63	0.43	0.67	0.53	0.20
LDH/C	1.82	0.49	2.18	1.52	1.07	5.68	3.23	0.37	0.80	0.80	1.30	1.99	2.48
αHBDH/C	1.86	N.D.	2.05	0.86	0.80	2.84	2.42	0.37	N.D.	0.80	1.30	1.33	1.92
Microp./C	0.24	0.32	0.13	0.50	0.32	0.69	0.20	0.05	0.18	0.17	0.13	0.15	0.07
C	12.81	10.16	24.30	23.00	18.56	1.76	6.18	13.26	6.18	6.18	11.49	15.02	17.68

Enzymes: U/L; MICROPROTEINURIA mg/dl; CREATININURIA (C) mmol/L
N.D. = Not detectable

Table 4. Values of urinary enzymatic activities and microproteinuria in the third class.
- Macroscopic report: both kidneys FOC++
- Histologic report: both kidneys NIC++

	$\bar{X}$	1	2	3	4	5	6	7
NAG/C	0.30	0.06	0.11	0.15	0.97	0.36	0.13	0.30
AP/C	3.07	N.D.	0.23	0.22	10.11	6.13	0.81	0.92
γGT/C	1.36	0.90	0.29	0.55	3.86	2.61	0.35	0.88
LDH/C	3.69	3.39	1.30	1.13	11.36	5.68	0.47	2.56
αHBDH/C	3.69	N.D.	N.D.	N.D.	5.68	5.68	0.47	1.13
Microp./C	0.55	0.04	0.06	0.10	0.91	0.30	0.09	2.40
C	9.58	4.42	23.80	13.26	0.88	0.88	10.60	13.26

ENZYMES: U/L; MICRPROTEINURIA mg/dl;
CREATININURIA (C): mmol/L
N.D. = Not detectable

Table 5. Values of urinary enzymatic activities and microproteinuria in the fourth class.
- Macroscopic report: both kidneys FOC+++
- Microscopic report: both kidneys NIC+++

	$\bar{X}$	1	2	3	4	5
NAG/C	0.46	0.92	0.18	0.14	0.86	0.22
AP/C	1.13	1.21	N.D.	N.D.	1.69	0.51
γGT/C	0.73	1.11	0.28	0.68	1.58	N.D.
LDH/C	2.77	5.56	1.88	2.05	3.77	0.62
αHBDH/C	1.57	3.23	0.62	1.54	1.88	0.62
Microp. /C	0.28	0.35	0.35	0.23	0.31	0.18
C	6.89	6.18	7.95	9.72	2.65	7.95

ENZYMES: U/L; Microproteinuria; mg/dl;
Creatininuria (C): mmol/L
N.D. = Not detectable

Table 6. Values of urinary activities and microproteinuria of the subjects with:

1. Renal amyloidosis
2. Lobular old infarction
3. Lobular acute infarction
4. Chronic interstitial nephritis
5. Pan-nephritis in cattle with pleuro-pneumonia

	1	2	3	4	5
NAG/C	1.58	0.20	2.73	0.01	1.37
AP/C	0.83	N.D.	1.20	1.30	N.D.
GT/C	1.22	0.67	0.44	0.78	0.91
LDH/C	1.41	1.13	1.54	1.13	2.82
HBDH/C	N.D.	N.D.	0.51	0.56	0.70
Microp. /C	3.18	0.08	2.10	0.08	5.30
C	10.60	4.42	9.70	8.84	7.07

ENZYMES: U/L; MICROPROTEINURIA; mg/dl; CREATININURIA (C): mmol/L
N.D. = Not detectable

Abstract

Effects on cattle of transport by road for up to 15 hours

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Twenty-four castrated male cattle aged between 12 and 18 months were transported by road for five, 10 or 15 hours, over distances of 286, 536 and 738 km. Half the animals were of Hereford x Friesian breeding and half of 'continental' type. The animals transported for five hours lost 4.6 per cent of their bodyweight, those transported for 10 hours lost 6.5 per cent and those transported for 15 hours lost 7.0 per cent; recovery to pre-transport values took five days. There was little evidence from changes in blood composition that a 15 hour journey was more stressful than a 10-hour journey. The cortisol concentrations were increased by the stresses of loading and the first part of the journey but then recovered as the journey continued. Creatine phosphokinase (CPK) activities increased progressively with the longer journeys and CPK, urea, albumin and osmolality levels recovered more slowly after the longer journeys. Increases in free fatty acids, beta-hydroxybutyrate and urea concentrations and the continued increase in urea levels after the end of the journeys suggested that the animals' normal pattern of feeding was disrupted. Increases in albumin, total plasma protein and osmolality indicated slight dehydration during transit which was quickly rectified by access to water. The two breed types responded similarly to transport, except that the increases in CPK were greater muscularity or greater sensitivity to stress. Based on the physiological measurements made and the subjective observations of behavior a 15-hour transport period under good conditions is not unacceptable from the viewpoint of animal welfare.