Blood serum concentrations of total proteins and main protein fractions in weaning rabbits experimentally infected with E. coli

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SUMMARY

The objective of the present study was to evaluate the changes in the concentrations of major blood proteins associated with experimental E. coli infection in weaning rabbits. For that, in the assay group, 12 weaning White New Zealand rabbits (45 days old) were orally infected with a bacterial suspension of enteropathogenic E. coli strain type O15:H-(6.10^7 cfu) whereas the 6 control rabbits received only 0.9% NaCl solution. Serum total protein, albumin, globulin and lysozyme concentrations as well as plasma fibrinogen concentrations were measured before (0h) and 1, 3, 7, 11, 18 and 30 days after oral treatment. In parallel, presence of coliforms was investigated in rectal samples on days 0, 1, 6, 11, 16, 25 and 30. Infected rabbits began to excrete E. coli strains on day 2 after administration, whereas the first signs of diarrhoea were observed on day 5. Between days 11 and 18 severe diarrhoea was found in all rabbits and then clinical signs gradually disappeared although 3 rabbits continue to excrete the bacteria on day 30. In inoculated rabbits, hypoproteinemia and hypo-albuminemia compared to control values were evidenced since the 7th day whereas the blood concentrations of lysozyme and fibrinogen at a lesser extend were dramatically increased on days 11-18 and on days 3-18 respectively, leading to a significantly lowered albumin/globulin ratio since the 11th day. These results confirm that albumin is a negative acute phase protein (APP) while fibrinogen and lysozyme were 2 positive APP in response to an experimental bacterial infection in rabbits.

Keywords: Total protein, protein fractions, lysozyme, fibrinogen, blood, rabbit, E.coli infection.


Introduction

Proteins are present in all body fluids, but the blood plasma proteins are examined most frequently for diagnostic purposes. Over one hundred individual proteins have a physiological function (transport, humoral immunity, maintenance of oncotic pressure, enzymes, protease inhibitors, buffering) in the plasma. Principal plasma proteins are albumin (Alb) and prealbumin; α1-globulins (α1-antitrypsin, α1-acid glycoprotein), α2-globulin (haptoglobin, α2-macroglobulin, ceruloplasmin); β-globulin (transferrin, low density lipoproteins, C3) and γ-globulins (fibrinogen and immunoglobulins) [2, 4, 24].

Albumin is a large, osmotically active protein with an average molecular weight of 69 kDa and a half-life between 17 and 19 days [11]. Globulins have molecular weights ranging from 90 kDa (β1-globulins) to 156 kDa (γ-globulins) [11]. Serum globulin concentration is determined by subtracting the albumin concentration from the total serum protein

Mots-clés : Protéines totales, fractions protéiniques, lysozyme, fibrinogène, sang, lapin, infection à E. coli.

concentration [11]. Fibrinogen is produced by the liver and plays an important role in the coagulation pathway [2, 12, 13].

Changes in serum protein concentrations result in a variety of clinical signs and systemic effects and are associated with a number of disease processes [11]. Acute phase proteins (APPs), approximately 40, such as α1-antitrypsin, α1-acid glycoprotein, haptoglobin, α2-macroglobulin, ceruloplasmin, transferrin, fibrinogen, C-reactive protein, serum amyloid A, tumour necrosis factors, are serum proteins whose circulating concentrations vary during the acute phase response to inflammation or infection and they play important defensive roles [2, 9, 13]. Their expression represents some effector mechanisms of innate immunity. The circulating concentration of some APPs rise and these proteins are called positive APPs, whereas in the case of negative APPs, their serum concentrations decrease [2]. These remarkable APPs have been extensively studied in humans and in laboratory animals [10, 22, 28].

The response to infection and other traumas involves a large number of changes and leads to a wide-ranging APP response in acute inflammation. Within the literature there are few systematic data concerning APP variations induced by bacteria in rabbits, but variations of the major plasma protein concentrations after inflammatory process are poorly investigated in rabbits [22, 26, 27].

During recent years, the interest has been increasingly focused on diarrhoea in weaning rabbits, since this condition has been responsible for major losses in the large rabbit farms. The investigations of PROHASZKA and BARON [29] revealed that weaning diarrhoea occurred exclusively in stock fed with a commercial diet and outbreaks of rabbit diarrhoea occurred always during the first two weeks after weaning, when the animals were 5-7 weeks old. PROHASZKA [30] found that E.coli diarrhoea is a typical weaning disease as rabbits generally develop it between 5 and 8 weeks of age in condition of large scale farming.

The present study was conducted to evaluate the changes in the concentrations of total serum protein (TP) and major proteins, i.e. albumin (Alb) and globulins (Glb) as well as lysozyme (Lsz) as part of the innate immune system, and plasma fibrinogen (Fb) during experimentally induced E.coli infection in weaning rabbits.

Materials and Methods

ANIMALS AND STUDY DESIGN

The experiments were carried out on 18 White New Zealand, 40-45 days old rabbits. The animals were weaned when they were 30 day old and the examination of their faeces ruled out the presence of Eimeria oocysts and E. coli. The rabbits were housed (4 animals per cage) in disinfected metal cages with a slat floor and they were exposed to a 12h light-dark cycle at room temperature (20-22°C). They were fed ad libitum with a commercially available diet of rabbit pellet without coccidiostatic and had free access to water.

When they were 45 day old, rabbits were randomly constituted: the experimental group with the infected rabbits (n = 12) and the control group with not infected rabbits (n = 6). Rabbits of the experimental group were orally infected with a bacterial suspension of enteropathogenic E. coli U83/39 (O15:H-, kindly provided by Dr. J.E. PEETERS, National Institute of Veterinary Research, Brussels, Belgium) (2 mL, 6.107 cfu) using a sterile non pyrogenic feeding tube (2.0x3.0mm2/25cm). In the same way, only the vehicle 0.9% NaCl solution (2 mL) was administrated to the control rabbits.

Blood samples from each rabbit were taken from v. auricularis externa prior to (0h) and 1, 3, 7, 11, 18 and 30 days after the E.coli or vehicle administration. Tubes were kept at room temperature for 2h to allow clotting, then they were centrifuged 2 000 g at 4°C for10 minutes and serum was decanted and stored at -20°C until assayed. For the determination of fibrinogen blood samples were taken in heparinized tubes and were immediately centrifuged to obtain plasma. Plasma samples were also stored at -20°C until assayed.

Rectal samples were obtained from all animals prior to the coliform infection. Similarly, discharge of the challenge strain was investigated on days 1, 6, 11, 16, 25 and 30 post-inoculation. The presence of coliforms was also investigated in the content of small intestines, in bowels and in caecum from dead rabbits.

ANALYTICAL METHODS

All samples investigated for the occurrence of Enterobacteriaceae were cultivated aerobically on MacConkey agar (Difco) at 37°C for 24 hours. The identification of enteropathogenic E.coli (EPEC) re-isolates was done by the semi-automated system Crystal (Becton Dickinson). The serotyping with a specific anti O15 antiserum (BulBio, National Centre of Infection and Parasitic Diseases, Sofia, Bulgaria) was done by slide agglutination using the method of GRUBER [in 31].

The total serum protein concentrations were measured by the biuret method [21]. Serum albumin concentrations were determined by the Bromcrezol green method using a commercial human kit (SU-ALBU INF 156001F, Gesellschaft fur Biochemica, Germany). Plasma fibrinogen concentrations were determined by the nephelometric method according to PODMORE [45]. Globulin concentrations were determined by subtracting the albumin concentrations from the total protein concentration. Lysozyme concentration was measured by the method of LIE and SYED [23] on the basis of radial immunodiffusion in agarose gel.

STATISTICAL ANALYSIS

All data are expressed as mean ± standard deviation (SD). The statistical analysis of the data was performed using one way analysis of variance (ANOVA). The significance of differences of means between post infection and base line values and between experimental and control groups for the same time were evaluated by the Bonferroni test. Differences were considered as significant when p values were less than 0.05.
Results

CLINICAL AND BACTERIOLOGICAL FINDINGS

Bacteriological examinations of rectal samples confirmed that all isolates from infected animals belonged to the type 015: H-E. coli strain used for the experimental infection. Three of the infected rabbits began to excrete the E. coli strain 2 days after bacteriologic administration. On day 3, all infected rabbits except one excreted the 015: H- E. coli strain in the faeces. The number of the colonies varied from single on day 2 to solid and dense growth on day 4 onward. Clinical signs of diarrhoea firstly appeared in two rabbits on day 5. Until the day 13, severe diarrhoea syndrome was observed in 6 rabbits and in all rabbits on the day 14 (n = 12), whereas on day 18 clinical signs of intense disease were found in the rest 8 rabbits. Four animals were severely affected and were slaughtered by intravenous injection of Na-pentobarbital (100 mg/kg) between days 14 and 17. Later, clinical signs of diarrhoea diminished and no diarrhoea was observed in all remained rabbits on day 30 (n = 8). Bacteriological examinations of rectal samples on day 30 showed that only two rabbits continued to excrete single colonies of the E. coli type 015:H- strain in the faeces.

BLOOD SERUM / PLASMA PROTEIN CONCENTRATIONS

The total serum protein concentrations (figure 1) and serum albumin concentrations (figure 2) gradually increased according to time in the control rabbits; the total serum protein concentrations became significantly higher than initial values on days 7, 11, 18 and 30 (p < 0.001) and serum albumin concentrations reached maximal values on days 18 and 30 (p < 0.001) in the control rabbits. By contrast, the variations of total serum protein concentrations in experimental rabbits remained weak during the same period although total protein concentrations tended to increase at the end of the experiment (on days 18 and 30) (figure 1). Consequently, total protein measurement was significantly lowered compared to control values since the 7th day (p < 0.001). In the same way, after a weak increase from day 0 to day 3, albumin concentrations tended to decrease on days 7 and 11 and then remained relatively constant (figure 2); they were significantly diminished compared to control values on days 7 (p < 0.05), 11 (p < 0.01), 18 and 30 (p < 0.001).

The variations of the globulin concentrations according to time were quite different in the control and infected rabbits (figure 3). Whereas they dramatically increased during the first days (from day 0 to day 3), rapidly decreased on day 7 and thereafter remained roughly constant in not infected animals, globulin concentrations poorly fluctuated until the 3rd day and thereafter gradually increased in the experimental group. Nevertheless, no statistically significant differences in globulin concentrations were evidenced between control and infected rabbits because of the great value dispersion observed in both 2 groups. Consequently, the albumin/globulin (A/G) ratio peaked in the experimental group on day 3 and then markedly decreased on day 7 whereas it felt the 3rd day and increased on day 7 for reaching maximal values on days 18 and 30 in the not infected animals (experimental group vs. control group: p < 0.05 on days 11, 18 and 30) (figure 4).

The plasma fibrinogen (figure 5) and serum lysozyme (figure 6) concentrations remained relatively stable in the control group except on the day 11 when maximal fibrinogen concentrations were recorded (day 11 vs. day 0: p < 0.001). By contrast, plasma fibrinogen concentrations dramatically increased since the 3rd day in the experimental group, peaked on day 11 (day 3 vs. day 0: p < 0.01; day 7 and day 11 vs. day 0: p < 0.001; experimental group vs. control group: p < 0.001 on days 3 and 11, p < 0.05 on day 7) and then gradually decreased for reaching pre-treatment values on day 30. In parallel, the serum lysozyme concentrations in experimental group progressively enhanced 3 and 7 days after E. coli administration, reached maximal values on day 11 and remained markedly elevated until the 18th day compared to the initial values (p < 0.001 on day 11, p < 0.05 on day 18) and to pre-initial values obtained in the experimental group at the same dates (p < 0.001). The elevations of the serum lysozyme concentrations induced by the E. coli infection was remarkable, the values recorded on the day 11 being 12 fold higher than initial values.
Discussion

The results of the present study further elucidate the behaviour of major blood proteins in response to infection in rabbits. The concentrations of total serum proteins and of particular blood proteins (i.e. fibrinogen and lysozyme) were variably affected by the *E. coli* administration. In inoculated rabbits, while hypoproteinemia and hypo-albuminemia compared to control values were evidenced since the 7th day when the diarrhoea syndrome occurred, blood globulin concentrations and essentially the albumin/globulin ratio as well as the fibrinogen and lysozyme concentrations markedly varied within the experimental period. The A/G ratio was significantly lowered on days 11-30 probably because of the dramatic increase of the lysozyme concentrations and of fibrinogen concentrations at a lesser extend.

The serum albumin concentrations measured in control rabbits in the present study were closely related to values previously observed by STEZENIC (in [33]) in adult rabbits (43 ± 0.5 g/L). The observed decrease of serum albumin concentrations in infected rabbits from the 7th day to the 30th day was in agreement with the study of ORHUE *et al*. [25]: they reported a significant diminution of albuminemia 14 days after *Trypanosoma brucei*-infection in rabbits probably due to impaired liver synthesis and /or losses via the gut, kidney or both. Consequently, albumin can be considered as a negative APP in mammalian species [3-5, 8, 18, 19].

According to SCHREIBER *et al*. [33], the serum total protein concentrations remained fairly constant 6 days after the induction of an inflammation process. In the present study, any significant variation of this parameter compared to controls was not recorded in *E. coli* infected animals before the 7th day. From their experiment, SCHREIBER *et al*. [33] demonstrated that the leucin content of serum total proteins varied between healthy rats and rats with inflammation (10.1% and 9.9% respectively) and that the albumin synthesis rate was markedly reduced during inflammation (91 mg of neo-synthesized albumin / 100 mg of body weight / day in healthy rats vs. 32 mg / 100 mg / day in rats with inflammation). Assuming that the same leucin pool is used for all serum protein synthesis, they found that the synthesis rates of the serum total proteins were 234 and 380 mg / 100 mg /day in control rabbits and animals with inflammation respectively. Consequently, the overall liver protein synthesis was only
moderately modified during inflammation despite the extraordinary increase of some positive APP synthesis rates. The increased consumption of translation tools (aminoacyl-tRNAs, GTP, ATP…) may be compensated by the simultaneously decreased synthesis of some negative APPs (albumin, transferrin, transthyretin, insulin-like growth factors…) not important for the host defence [1, 2, 27]. Albumin is the most appropriate for this metabolic adaptation: its half-life is very long, its total body pool is the largest among the plasma proteins and it has no specific indispensable function. On the other hand, the amino acids necessary for positive APP synthesis may be also supplied throughout the ubiquitin dependent catabolism of muscle proteins [5].

As positive APPs are globulins, the easy determination of the serum globulin concentrations may contribute to the detection of an inflammatory process [1, 2, 14-16]. Although this parameter tended to increase in the E. coli infected rabbits from the 7th to the 30th days (probably because of the decrease of albumin concentrations in parallel), the differences with control values at the same dates were not significant because of the great value dispersion. There was an increase of serum globulin concentration at the 3rd day in control group; however, the differences compared to the initial values were not statistically significant. According to BURTIS [4], STOYANCHEV [42] and STOYANCHEV et al. [43] increased immunoglobulin concentrations are seen in both acute and chronic infections and the measurement of the specific immunoglobulin class concentration does not supply any positive diagnostic gain.

The plasma fibrinogen concentrations began to significantly increase in infected rabbits compared to the controls since the 3rd day, indicating that fibrinogen could be classified as slow reacting positive APPs in rabbits in response to bacterial infection. But, because the magnitude of these increases (between 43% and 97%) remained moderate, this protein is considered as a minor positive APP in agreement with previous studies [6, 9, 13, 32, 33].

The role of lysozyme in host defence against infection remains relatively elusive [7] although elevated circulating lysozyme concentrations were evidenced after antigen stimulation [17, 20, 34-41, 44]. In the same way, PAWLIKOWSKA and DEPTULA [27] reported that lysozyme concentrations increased before the efficient production of specific antibodies. In the present study, E. coli infected rabbits exhibited considerable increases of the serum lysozyme concentrations on days 11 and 18, confirming the role of this protein in the innate immunity and that lysozyme can be considered as a late positive APP.

In conclusion, the observed variations of blood albumin and globulin (fibrinogen and lysozyme) concentrations showed that fibrinogen was a slow reacting minor positive APP, lysozyme a late major positive APP while albumin was a negative APP in rabbits in response to an experimental bacterial infection. Besides, the serum total protein concentration may be a reliable and relatively precocious marker for the early detection of bacterial infections in weaning rabbits before the appearance of clinical signs of diarrhoea.

References

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