



International Journal of Pharmacology

ISSN 1811-7775

science
alert

ansinet
Asian Network for Scientific Information

Effect of Administration of Fungal Mycotoxin (Gliotoxin) on Clinical and Serobiochemical Parameters in Camels

M.S. Shathele

Department of Microbiology, King Faisal University, P.O. Box 1757,
Al-Ahsa 31982, Kingdom of Saudi Arabia

Abstract: The main objective of this study was to determine the effect of administration of fungal mycotoxin (Gliotoxin) on clinical and serobiochemical parameters in camels. A bolus of $0.1 \mu\text{g kg}^{-1}$ b.wt. of gliotoxin was administered intravenously to camels. The treated camels were lethargic and decreased their appetite from day 3 onwards. The toxin administration decreased the protein and glucose concentration of serum. The increased activity of aspartate amino transferase, gamma glutamyl-transferase, sorbitol dehydrogenase and alkaline phosphatase due to mycotoxin administration was indicative of liver damage. High concentration of urea nitrogen and creatinine in treated animals was a characteristic of renal injury. In conclusion, the gliotoxin is acutely toxic to camels affecting liver and kidney function.

Key words: Camels, gliotoxin, serum, hepatotoxic, nephrotoxic, protein, glucose, liver, kidney

INTRODUCTION

Gliotoxin is a mycotoxin produced by a number of fungi including important human and animal pathogens as *Aspergillus fumigatus* (Bauer, 1994; Eichner *et al.*, 1988). Gliotoxin affects the rumen fermentation, reducing digestibility and volatile fatty acid production *in vitro* (Morgavi *et al.*, 2004). Reeves *et al.* (2004) using an insect model demonstrated the significance of gliotoxin in increasing the virulence of *A. fumigatus*. Gliotoxin is a well studied non-ribosomal peptide toxin (Gardiner and Howlett, 2005) and has long been fingered as a putative factor contributing to invasive aspergillosis due to its cytotoxic (Grovel *et al.*, 2002), genotoxic (Nieminen *et al.*, 2002) and apoptotic properties (Hesseltine, 1986; Kweon *et al.*, 2003; Niyo *et al.*, 1988; Pahl *et al.*, 1996; Schiefer, 1990). Presently, the difficulty of diagnosis increased due to limited research, occurrence of multiple mycotoxins, non-uniform distribution, interactions with other factors and problems of sampling and analysis. Because of the difficulty of diagnosis, the determination of a mycotoxin problem becomes a process of elimination and association. This study was designed to investigate the clinical and serobiochemical effects induced by gliotoxin in camels in an effort to diagnose the fungal infection in camels.

MATERIALS AND METHODS

Ten healthy and mature (3-4 years old having 200-250 kg b.wt.) camels were used in the study. They

were kept in an open yard with free access to food and water. Animals were divided randomly into two groups. Group A animals were treated with saline and kept as controls. Group B animals were injected with gliotoxin (Sigma Chemicals, UK). In a pilot experiment, the dose of $0.2 \mu\text{g kg}^{-1}$ b.wt., administered intravenously (IV) killed all the three animals tested. Therefore, a bolus of $0.1 \mu\text{g kg}^{-1}$ b.wt. was administered IV to group B animals. The blood samples were collected daily for seven days into plain tubes. Serum was separated and stored at -30°C until analysis.

Serum biochemical analysis: The serum was analyzed for total protein, urea nitrogen, creatinine, glucose, total bilirubin, aspartate amino-transferase (AST), Gamma Glutamyl Transferase (GGT), alkaline phosphatase (ALKP), Sorbitol Dehydrogenase (SD) and Lactic Dehydrogenase (LD) by automated methods (Hitachi 704 Automatic Analyzer, Hitachi, Tokyo, Japan). Anion gap was quantified and calculated as $= [\text{Na}] + [\text{K}] - ([\text{Cl}] + [\text{Hco}_3])$ (Constable *et al.*, 1997).

RESULTS

The administration of gliotoxin to camels produced anorexia and the animals were depressed and lethargic after day 4 of administration. The serobiochemical profiles of camels administered with gliotoxin are shown in Table 1. The sampling day and treatment interactions were detected ($p < 0.01$) from day 2 of administration for all parameters except creatinine, protein, urea nitrogen and

Table 1: Effect of administration of gliotoxin (0.1 µg kg⁻¹) on some serobiochemical parameters in camels

Parameter	Control group A ⁽ⁿ⁼⁵⁾	Time (days) after injection of gliotoxin in group B ⁽ⁿ⁼⁵⁾						
		1	2	3	4	5	6	7
Total protein (g dL ⁻¹)	5.4±0.4	5.3±0.3	5.0±0.4	4.2±0.2	4.2±0.2	3.2±0.1*	3.4±0.1*	3.3±0.1*
Urea nitrogen (mg dL ⁻¹)	13.2±1.2	12.1±1.3	14.4±1.4	15.4±0.8	16.3±0.7*	17.6±0.7*	16.4±0.7*	16.12±0.8*
Creatinine (mg dL ⁻¹)	0.80±0.03	0.79±0.03	0.81±0.03	1.0±0.04*	1.1±0.04*	1.3±0.4*	1.4±0.05*	2±0.06*
Glucose (mg dL ⁻¹)	118.2±12.1	106±10	80±8*	75±7*	70±7*	72±6*	68±6*	60±5*
Total bilirubin (mg dL ⁻¹)	0.6±0.04	0.8±0.06	2.1±0.2*	3.2±0.4*	4.1±0.6*	5.4±0.5*	6.1±10.6*	5.6±0.6*
Aspartate aminotransferase (µ L ⁻¹)	56±3	56±3	80±4*	90±5*	100±6*	130±10*	180±12*	200±14*
Gamma-glutamyl-transferase (µ L ⁻¹)	180±12	170±11	190±12	200±14	205±15*	220±16*	225±17*	230±18*
Sorbitol dehydrogenase (µ L ⁻¹)	6±0.4	10±1	16±2*	25±3*	50±4*	60±5*	55±5*	50±6*
Alkaline phosphatase (µ L ⁻¹)	220±14	230±14	270±16*	460±18*	600±21*	700±20*	750±22*	1000±25
Lactic dehydrogenase (µ L ⁻¹)	133±11	140±12	130±12	135±13	146±12	140±12	130±12*	135±14*
Anion gap (mEq L ⁻¹)	14±2	16±2	18±2*	19±3*	20±3*	22±4*	23±4*	24.4±4*

*p<0.01, significantly different from controls

lactic dehydrogenase. The effect of gliotoxin administration was observed on day 3 for creatinine, on day 4 for urea nitrogen and on day 5 for protein. However, no effect was observed on the activity of LD. Higher concentration levels of urea nitrogen, Creatinine, total bilirubin and anion group (p<0.01) were observed in group B as compared to group A animals. Also, increased activity (p<0.01) of ASP, GGT, SD and ALKP was found in group B than group A animals. A decrease (p<0.01) in concentration of protein and glucose was observed in group B than group A animals.

DISCUSSION

It was found in this study that the camels administered with gliotoxin were lethargic and anorexic. They were also depressed from day 3 until day 7. The gliotoxin has been proved to be toxic to a number of animal species *in vivo* (Eichner *et al.*, 1988; Bauer, 1994) and *in vitro* (Morgavi *et al.*, 2004). The serum protein concentration decreased on day 5 after administration but the serum glucose concentration decreased at a faster rate in gliotoxin treated animals. This effect might be due to decrease in appetite and suggests that the treated animals were in a state of negative energy balance. Animals treated with gliotoxin maintained lower serum glucose even in the pre-diabetic state (Larsen *et al.*, 2000). However, animals treated chronically with small doses of gliotoxin showed an increase in weight gains as compared to those in controls (Larsen *et al.*, 2000). An increase in serum bilirubin in animals treated with gliotoxin suggested hepatic dysfunction (Cornelius, 1987). Serum activities of AST, GGT, SD and ALKP were also higher in these animals thus reflecting damage to the liver. The AST is present in high concentration in muscle, liver and brain and the serum elevation indicates damage to one or more of their tissues (Murray *et al.*, 1990). Elevation of GGT, SD and ALKP activities are indices of damaged liver and obstruction of the biliary tract (Cornelius, 1989). Similar increases in liver enzymes were reported in calves

(Osweiler *et al.*, 1993), lambs (Edrington *et al.*, 1995), swine (Colvin *et al.*, 1993), horses (Ross *et al.*, 1993) and broiler chicks (Ledoux *et al.*, 1992) intoxicated with another mycotoxin fumonisin. The concentration of both the urea nitrogen and creatinine in serum increased in gliotoxin treated animals. Elevations of both metabolites were observed in cases of acute renal insufficiency and urinary tract obstruction (Murray *et al.*, 1990). However, a few researchers have reported an increase in these indices in case of decreased plasma volume, renal blood flow and glomerular blood flow (Smith *et al.*, 1999; Constable *et al.*, 2000). The anion gap also increased in treated camels from day 3 onward reflecting metabolic acidosis (Mathur *et al.*, 2001). Such effects may also be due to the presence of anions associated with uremia (Constable, 1997).

CONCLUSIONS

The gliotoxin administrated IV at a dose of 0.1 µg kg⁻¹ b.wt. proved to be hepatotoxic and nephrotoxic in camels. The study showed an excellent potential for future investigations to determine the effect of chronic administration of smaller doses of gliotoxin to camels which may occur under natural exposure.

ACKNOWLEDGMENTS

The author is thankful to the deanship of Scientific Research for financial support.

REFERENCES

- Bauer, J., 1994. Möglichkeiten zur entgiftung mykotoxinhaltiger futtermittel. Montash Vet. Med., 49: 175-181.
- Colvin, B.M., A.J. Cooleu and R.W. Beaver, 1993. Fumonisin toxicosis in swine: Clinical and pathological findings. J. Vet. Diagn. Invest., 5: 413-417.

- Constable, P.D., R.N. Streeter, G.J. Koeing, N.R. Perkins, H.M. Gohar and D.E. Mortin, 1997. Determinants and utility of the anion gap in predicting hyperlactemia in cattle. *J. Vet. Inter. Med.*, 11: 71-79.
- Constable, P.D., G.W. Smith, G.E. Rottinghaus and W.M. Haschek, 2000. Ingestion of fumonisin B1 containing culture material decreases cardiac contractility and mechanical efficiency in swine. *Toxicol. Applied Pharmacol.*, 162: 151-160.
- Cornelius, C.E., 1987. A review of new approaches to assessing hepatic function in animals. *Vet. Res. Commun.*, 11: 423-441.
- Cornelius, C.E., 1989. Liver Function. In: *Clinical Biochemistry of Domestic Animals*, Kaneko, J.J. (Ed.). Academic Press, San Diego CA., pp: 364-397.
- Edrington, T.S., C.A. Kamps-holtzapfel, R.B. Harvey, L.F. Kubena, M.H. Elissalde and G.E. Rottinghaus, 1995. Acute hepatic and renal toxicity in lambs dosed with fumonisin containing culture material. *J. Anim. Sci.*, 73: 508-515.
- Eichner, R.D., U.T. Palni, P. Waring and A. Mullbacher, 1988. Detection of the immunomodulating agent gliotoxin in experimental aspergillosis. *Proceedings of the Tenth Congress of International Society Human and Animal Mycology, (TCISHAM'88)*, Barcelona, pp: 133-137.
- Gardiner, D.M. and B.J. Howlett, 2005. Bioinformatic and expression analysis of the putative gliotoxin biosynthetic gene cluster of *Aspergillus fumigatus*. *FEMS Microbiol. Lett.*, 248: 241-248.
- Grovel, O., Y.F. Pouches, T.R. du Pont, M. Montagu, Z. Amzil and J. Verbist, 2002. Ion trap MS (n) for identification of gliotoxin as the cytotoxic factor of a marine strain of *Aspergillus fumigatus* Fresenius. *J. Microbiol. Methods*, 48: 171-179.
- Hesseltine, C.W., 1986. Resume and Future Needs on the Diagnosis of Mycotoxins. In: *Diagnosis of Mycotoxicosis*, Richard, J.L. and J.R. Thurston (Eds.). Martinus Nijhoff Publishers, Dordrecht, The Netherlands, pp: 381-385.
- Kweon, Y.O., Y.H. Paik, B. Schanbal, T. Qian, J.J. Lemasters and D.A. Brenner, 2003. Gliotoxin-mediated apoptosis of activated human hepatic stellate cells. *J. Hepatol.*, 39: 38-46.
- Larsen, B., H. Liu, S. Jackman and H. Driscoll, 2000. Effect of gliotoxin on development of diabetes mellitus in diabetes-prone BB/Wor rats. *Ann. Clin. Lab. Sci.*, 30: 99-106.
- Ledoux, D.R., T.P. Brown, T.S. Weibking and G.E. Rottingham, 1992. Fumonisin toxicity in broiler chicks. *J. Vet. Diagn. Invest.*, 4: 330-337.
- Mathur, S., P.D. Constable, R.M. Eppley, A.L. Waggoner, M.E. Tumbleson and W.M. Haschek, 2001. Fumonisin B1 is hepatotoxic and nephrotoxic in milk fed calves. *Toxicological Sci.*, 60: 385-396.
- Morgavi, D.P., H. Boudra, J.P. Jouany and B. Michalet-Doreau, 2004. Effect and stability of gliotoxin an *Aspergillus fumigatus* toxin on *in vitro* rumen fermentation. *Food Additives Contam.*, 21: 871-878.
- Murray, R.K., D.K. Granner, P.A. Mayes and V.W. Rodwell, 1990. *Harpers Biochemistry*. 22nd Edn., Appleton and Lange, East Norwalk, CT.
- Nieminen, S.M., J. Maki-Paakkanen, M.R. Hirvonen, M. Roponen and A. von Wright, 2002. Genotoxicity of gliotoxin a secondary metabolite of *Aspergillus fumigatus* in a battery of short term test systems. *Mutat. Res.*, 520: 161-170.
- Niyo, K.A., J.L. Richard, Y. Niyo and L.H. Tiffany, 1988. Effects of T-2 mycotoxin ingestion on phagocytosis of *Aspergillus fumigatus* conidia by rabbit alveolar macrophages and on hematologic, serum biochemical and pathologic changes in rabbits. *Am. J. Vet. Res.*, 49: 1766-1773.
- Osweiler, G.D., M.E. Kehrl, J.R. Stable, J.R. Thursten, F. Ross and T.M. Wilson, 1993. Effects of fumonisin contaminated corn screenings on growth and health of feeder calves. *J. Anim. Sci.*, 71: 459-465.
- Pahl, H.L., B. Krauss, K. Schulze-Osthoff, T. Decker and E.B. Traenckner, 1996. The immunosuppressive fungal metabolite gliotoxin specifically inhibits transcription factor NF- κ B. *J. Exp. Med.*, 183: 1829-1840.
- Reeves, E.P.C., G.M. Messina, S. Doyle and K. Kavanagh, 2004. Correlation between gliotoxin production and virulence of *Aspergillus fumigatus* in *Galleria Mellonella*. *Mycopathologia*, 158: 73-79.
- Ross, P.F., A.E. Ledet, D.L. Owens, L.G. Rice, H.A. Nelson, G.D. Osweiler and T.M. Wilson, 1993. Experimental equine leukoencephalomalacia, toxic hepatitis and encephalopathy caused by corn naturally contaminated with fumonisins. *J. Vet. Diagn. Invest.*, 5: 69-74.
- Schiefer, H.B., 1990. Mycotoxicosis of domestic animals and their diagnosis. *Can. J. Physiol. Pharmacol.*, 68: 987-990.
- Smith, G.W., P.D. Constable, M. Tumbelson, G.E. Rottinghaus and W.M. Haschek, 1999. Sequence of cardiovascular changes leading to pulmonary edema in swine fed culture material containing fumonisin. *Am. J. Vet. Res.*, 60: 1292-1300.