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In Vivo Toxicity of Concanavalin A[1] (38266)

MARVIN L. TYAN

Dental Research Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27514

Concanavalin A, Con A, is a carbohydrate-free metalloprotein containing Mn^{2+} isolated from the jack bean, *Canavalia ensiformis* (1). It agglutinates erythrocytes, embryonic cells and certain viruses and forms insoluble complexes with many polysaccharides and glycoproteins in a way analogous to the formation of antigen-antibody complexes. The reaction between Con A and carbohydrate containing molecules is specific for α-D-glucosyl and sterically related residues. Current interest in the reactions of Con A stems from its ability to agglutinate certain transformed and malignant cell lines (2), and from its action as a potent and specific mitogen for thymus-passaged lymphocytes (3).

While it has been recognized for many years that this protein can be toxic to animals when given orally or parenterally (4–7), to my knowledge, there is no published description of the pathology induced by Con A. Presented below are observations on the pathological changes encountered during an investigation of the immunosuppressive properties of this lectin (5, 7).

Materials and Methods. The experimental animals were adult male (C57BL/6 × DBA/2)F_1 (B6D2F_1), BALB/c, C3H/HeJ, and AKr mice. Concanavalin A, lot 89, was obtained from Miles Laboratories, Kankakee, IL. It was stored at −20° and dissolved in balanced salt solution just prior to use. Groups of mice were given 200, 400, or 800 μg Con A iv in 0.2 ml or 200, 400, 800, 1600, or 2000 μg ip in 0.5 ml. These groups were observed for 30 days for morbidity and mortality; 2, 7, 15, and 30 days after the injection of Con A from 2–6 B6D2F_1 mice were killed, autopsied, and selected tissues were fixed in 10% neutral formalin and H & E sections were prepared for study (8).

Results. No deaths occurred among the mice given 200 μg Con A iv; however, about 15% of the mice given 400 μg iv and a somewhat higher percentage of the mice given 800 μg died within 2 days (Table I). All mice tolerated the iv injection of Con A without distress; deaths occurred 12–48 hr after injection. All other mice appeared well throughout the period of observation. The data, although incomplete, suggest the possibility of strain variability in susceptibility to the toxic effects of Con A (B6D2F_1 > BALB/c > C3H > AKr). In contrast, the ip injection of Con A in doses up to 2000 μg produced no deaths and no apparent morbidity.

At autopsy 2 days after the injection of 200 μg Con A iv no gross pathological abnormalities were noted. Microscopically, occasional small areas of periportal necrosis infiltrated by minimal numbers of polymorphonuclear cells (PMN) were found in the livers. There was mild atrophy of the splenic white and red pulp associated with dilated sinusoids and small subcapsular hemorrhages. The subpleural aveolar capillaries were mildly congested. Five days later no significant gross or microscopic abnormalities were found.

Two days after the iv injection of 400 μg Con A all mice had gross evidence of

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TABLE I. Mortality Among Various Strains of Mice Given Con A iv or ip.

Con A (μg)	Route given	Mouse strain	Mortality[a] (No./total)
200	iv	B6D2F$_1$	0/10
400	iv	B6D2F$_1$	4/24
		BALB/c	1/7
800	iv	B6D2F$_1$	5/14
		C3H	2/14
		BALB/c	3/7
		AKr	0/7
2000	ip	B6D2F$_1$	0/14
		C3H	0/14
		BALB/c	0/7
		AKr	0/7

[a] All deaths occurred 12–48 hr after injection of Con A.

marked hepatic damage, mild splenic and thymic atrophy and hyperemic lungs. Microscopically, extensive areas of focal necrosis, central and periportal, were observed in the livers. These necrotic areas were primarily located adjacent to the hepatic capsule near the anterior edge, and they were infiltrated by moderate numbers of PMN. There was mild to marked atrophy of the splenic white and red pulp together with extensive subcapsular hemorrhaging from dilated sinusoids. The cortical areas of the thymus were mildly to moderately atrophic, and the pulmonary alveolar capillaries were congested.

Five days later, many fewer areas of hepatic necrosis were noted and regenerative processes were underway. The cortical areas of the thymus were hypertrophic at this time, while the microscopic picture in the spleen remained essentially unchanged except for the appearance of a moderate number of giant cells in the red pulp and for increased atrophy of the lymphoid areas. The subpleural aveolar capillaries remained moderately congested. Fifteen days after the injection of Con A the hepatic gross and microscopic findings were nearly normal. The splenic white pulp was slightly atrophic and mild cortical hyperplasia persisted in the thymus. The congestion noted in the pulmonary capillary bed remained essentially unchanged. Thirty days after injection no significant gross or microscopic abnormalities were noted.

Examination of the tissues of mice given Con A ip revealed local peritoneal inflammatory changes roughly proportional to the dose given. At the highest levels, 1600 and 2000 μg, large white peritoneal masses composed of amorphous material were found. There was no evidence of hepatic damage or splenic atrophy. It should be noted that Con A produces systemic immunodepression at all dose levels given ip in these experiments (5, 7).

Discussion. These studies confirm previous reports on the toxicity of intravenously administered Con A; 800 μg produced up to 30% mortality within 2 days in certain strains of mice and mild hepatic damage was induced by 200 μg. The most likely cause of death of these mice was acute hepatic failure, although other causes such as pulmonary insufficiency or overwhelming infection, while unlikely on clinical and pathological grounds, have not been ruled out. Injected intraperitoneally, Con A in doses up to 2000 μg produced local inflammatory changes but no deaths. Work from this and other laboratories (5, 7) have demonstrated systemic immunodepressant effects from Con A given ip; therefore this lectin is absorbed from the peritoneal cavities of mice although this may be both incomplete and prolonged.

The exact mechanism through which Con A expresses its toxicity remains unclear. It appears unlikely that it is a direct result of immunodepression with activation of latent viral or bacterial infections, because mice of the same strains severely immunosuppressed by a lethal dose of radiation (900 *r*) die 9–16 days later of marrow insufficiency and infection and without evidence of significant hepatic damage. However, direct activation by Con A of a latent hepatitis virus cannot be ruled out. Similarly, it is not possible to exclude a primary effect of Con A on hepatocytes or on vascular endothelial cells directly or as a result of lectin-induced RBC clumping and capillary occlusion. Additional studies are needed to clarify these points.

Summary. Con A administered intra-

venously in doses of 200–800 μg produced atrophy of lymphoid tissues, vascular endothelial damage and focal hepatic necrosis. At the highest dose given, up to 30% of the mice died within two days of what appeared to be acute hepatic failure. The injection of up to 2000 μg Con A ip produced only local inflammatory changes. The exact mechanism of Con A toxicity remains unclear.

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