classes[8]. Elimination of these agglutinins was achieved in this study by absorption with human AB and porcine erythrocytes, as evidenced by the extinction of haemagglutination reaction with simultaneous reduction in intensity of fluorescence. Thus, fluorescence seen after absorption will signify the presence of specific antibodies to the zona antigen in sera showing a strong positive reaction.

Although a causal relationship between the presence of autoantibodies and infertility in these women cannot be established at the moment, the facts that heteroantibodies to zonae of rodents can block fertilization in vitro[9-11] or in vivo[10,12] temporarily by passive transfer of the immune sera may suggest an involvement of the detected autoantibodies as an etiological factor in the development of infertility in these women. However, the question why autoantibodies are present in particular women remains unsolved, because all the women should have been equally exposed to the zona antigen and hence equally sensitized to it. One explanation is that the women with the autoantibody could be much more prone to autosensitization to the zona antigen than women without the antibody, and constant antigenic stimulation evoked through the physiological processes of atresia or ovulation could produce a higher titer of the antibody sufficient to develop infertility. This concept encourages us to utilize the zona antigen for immunological control of conception, because active immunization with this material could sensitize fertile women to produce sufficient antibody to inhibit fertilization. Further rigorous research is required to realize this system for human fertility control.

1 This work was supported in part by a grant from WHO Task Force on Immunological Methods for Fertility Regulation Project No. 76464.

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Long-term depression of two primary immune responses induced by a single dose of 5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide (DTIC)[1]

P. Puccetti, A. Giampietri and M.C. Fioretti

Institute of Pharmacology, University of Perugia, I-06100 Perugia (Italy), 26 October 1977

Summary. 2 primary immune responses (anti-SRBC antibody response and allograft rejection) have been tested in mice at various time intervals after single doses of either DTIC or cyclophosphamide. The DTIC-induced immunodepression proved to be extremely long-lasting, being still detectable after 2 months.

Among the most recent antitumor agents, 5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide (DTIC) has been the subject of extensive studies, recently reviewed[2], particularly since it was found to be highly active in mediating immunogenic changes of experimental murine lymphomas[3-8].

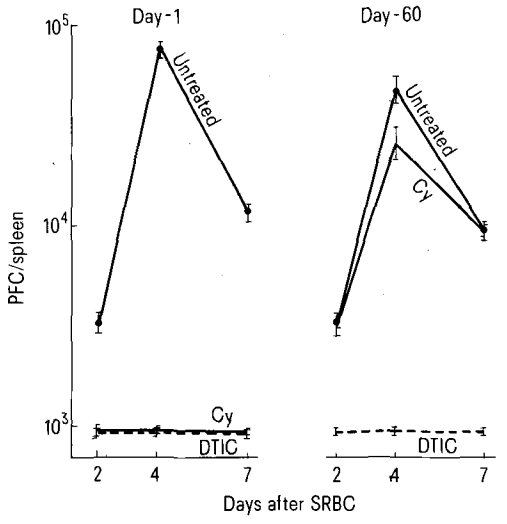
Preliminary observations in our laboratory showed unusual effects of DTIC on the immune responses. Therefore experiments were carried out to investigate how the immunodepressive activity of the drug compares to that of cyclophosphamide (Cy), a well-known alkylating agent widely employed as an experimental immunodepressant. The data demonstrate that a single dose of DTIC produced marked depression of both allograft and humoral antibody responses. The inhibitory effects were far more long-lasting than those induced by an equitoxic dose of Cy.

Materials and methods. 2 primary immune responses were tested in mice at various times after injection of single doses of either DTIC or Cy. The humoral antibody production was assayed against sheep red blood cells (SRBC) and the allograft response was measured against a mouse lymphoma line, which was transplanted across a histocompatibility barrier as strong as the entire H-2 complex. The humoral antibody production was assessed according to the method described by Jerne et al.[9], and results were expressed as number of primary hemolytic plaque-forming cells (PFC)/spleen. Mortality data, i.e. median survival time (MST) and number of dead mice over the total number of animals injected (D/T), have been used to measure the extent of allograft response in mice challenged with an allogeneic lymphoma.

Results. 2 4-month-old hybrid (DBA/2×BALB/c)F_1 ($CD2F_1$) mice of both sexes were treated i.p. with either DTIC 240 mg/kg or Cy 200 mg/kg. SRBC were administered 1 or 60 days later and the animals were then tested for their capability of producing humoral antibodies. Tests were performed on day 2, 4 and 7 after SRBC administration. The left side of the figure shows that both DTIC and Cy, given 1 day before SRBC injection, completely abrogated the humoral antibody production as measured by the number of PFC/spleen. When the drugs were administered 60 days before the anigenic stimulus (right side of the figure), only treatment with DTIC caused complete suppression of the antibody response. In a similar experimental design, the allograft response was tested in $CD2F_1$ (H-2^d/H-2^d) mice pretreated with either DTIC 240 mg/kg or Cy 200 mg/kg and then challenged i.v. with 10^6 allogeneic

Strain	Drug	Day	MST	D/T	Range
C57Bl/10	-	-	8	7/7	7-9
$CD2F_1$	-	-	-	0/6	-
$CD2F_1$	Cy	− 1	8	8/8	8-12
$CD2F_1$	DTIC	− 1	8	8/8	8-11
$CD2F_1$	Cy	−60	-	0/6	-
$CD2F_1$	DTIC	−60	10	6/6	9-10

Depression of the allograft response by equitoxic doses of 5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide (DTIC) and Cyclophosphamide (Cy) in mice challenged with the allogeneic lymphoma L5MF-22. MST, median survival time in days; D/T, dead over the total number of animals injected.



Effect of single equitoxic doses of DTIC and Cy on mouse antibody production. The drugs were given either 1 day (left side) or 60 days (right side) before SRBC and antibody production was assessed on day 2, 4 and 7 after the stimulus. ● ——— ● *Untreated;* ——— *Cy-treated animals;* -------- *DTIC-treated animals.*

L5MF-22 (H-2^b) cells 1 or 60 days later. The table shows that non-pretreated control $CD2F_1$ mice rejected tumor challenge, whereas animals pretreated with either DTIC or Cy 1 day before challenge died of generalized lymphoma with median survival times similar to those of compatible C57Bl/10 (H-2^b) mice. On the other hand, when drug treatment was performed 60 days earlier, the mice injected with Cy rejected the L5MF-22 tumor; in contrast $CD2F_1$ hosts pretreated with DTIC failed to reject the challenge and succumbed with generalized lymphoma.

Discussion. We have compared the immunodepressive activity of DTIC with that of Cy, an alkylating agent widely considered as a standard in experimental immunodepression. Primary antibody response and allograft reactivity have been examined, since previous studies[10,11] had shown that both are affected by DTIC treatment. Tests were performed at 2 different time intervals after drug treatment, 1 or 60 days, since previous observations had shown that a DTIC-induced depression of allograft response could exceed 20 days[2]. The remarkably long interval of 60 days was also chosen to rule out the possibility that the duration of the immunodepressive effects could be due to the persistence of DTIC in mouse organs after its administration. It has been repeatedly reported[13,14] that DTIC has a short halflife, as discussed elsewhere[2]. Consistent with previous reports[10,12], we have found that high equitoxic doses of DTIC and Cy abrogated the 2 primary immune responses under investigation, when the 2 drugs were given 1 day before the antigenic stimulus. But even when DTIC was given 60 days before the antigen, the immunodepression was still complete, the converse being true for Cy. The cellular bases of DTIC-induced immunodepression are still unknown. However, such long duration of the immunodepressive effects caused by DTIC suggests several hypotheses including the possibility that this agent might affect lymphoid cell populations (either suppressor or effector lymphocytes) with extremely long turnover rates. Moreover these findings suggest possible clinical applications of DTIC in the pharmacological control of graft rejection.

1 This work was supported by Project NO1-CB 64054 of the U.S.-Italy Cooperative Science Program.
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Inhibition of glycolysis by L-sorbose in dog erythrocytes

A. Kistler and P. Keller[1]

Biological Pharmaceutical Research Department, F. Hoffmann-La Roche & Co. Ltd, CH-4002 Basle (Switzerland), 11 November 1977

Summary. We have demonstrated previously that in vitro L-sorbose acts directly on dog erythrocytes to induce hemolysis. Here we report that L-sorbose depresses lactate formation in dog hemolysates from glucose, mannose and fructose but not from glucose-6-phosphate and galactose, suggesting that L-sorbose interacts with glycolysis at the level of the hexokinase.

Recently we reported that the ingestion of L-sorbose, a monosaccharide of the ketohexose group, causes hemolysis in dogs[2,3]. In vitro studies showed that L-sorbose acts directly on dog erythrocytes to induce hemolysis[4]. Sorbose concentrations as low as 2 mM lead to total hemolysis after 48 h of incubation. The dependence of the hemolytic effect of sorbose upon temperature and pH suggests that L-sorbose acts on the red blood cell metabolism rather than directly on the cell membrane[4].

The monosaccharide nature of L-sorbose would suggest an interaction with glycolysis. Since the energy available to erythrocytes is derived mainly from glycolysis, an inhibition of this metabolic pathway by L-sorbose could lead to a depletion of the ATP level and secondarily to hemolysis.

Materials and methods. Swiss Beagle dogs were obtained from the Institute of Biological and Medical Research (Füllinsdorf, BL). Blood samples were collected in heparinized tubes and the red blood cells washed 2-3 times with physiological NaCl solution. The red blood cells were incubated in Hanks balanced salt solution (with 20 mM Hepes-buffer, pH 7.4 instead of the bicarbonate buffer, without phenol red and supplemented with 60 µg/ml