Selective Toxicity of Amanitin-Albumin Conjugates for Macrophages

The toxicity of peptide amanitins for eukaryotic cells is a consequence of their inhibitory action on RNA polymerase II[1-3]. The conjugate obtained by coupling amanitin to bovine serum albumin (AMA-BSA) was found to damage selectively sinusoidal cells of the liver when injected into mice[4], and to kill in vitro peritoneal macrophages at much lower concentrations than those affecting lymphocytes and fibroblasts[5]. The sensitivity of liver sinusoidal cells and macrophages to AMA-BSA is most likely a consequence of the high protein uptake by pinocytosis displayed by these cells[6,7].

AMA-BSA may exert its toxic activity directly, because it inhibits RNA polymerase II in vitro[4], or by releasing amanitin after penetration into the cells. The latter mechanism is more likely because proteins are hydrolyzed by lysosomal enzymes after penetration into macrophages[7].

In these experiments we compared the sensitivity of peritoneal macrophages to AMA-BSA with that of several cells of different origins and characteristics.

AMA-BSA was prepared according to DERENZINI et al.[4]. The molar ratio of amanitin to albumin in the conjugate was found to be 1.9. Cells were incubated in Eagle's medium containing AMA-BSA or α-amanitin for 24 h at 37 °C. During the first 15 h of incubation, the medium did not contain serum[5]. Then inactivated foetal bovine serum was added to make a concentration of 10%; after further 9 h of incubation, the number of viable cells was determined by staining with trypan blue[18]. Macrophages were obtained from peritoneal exudate of Swiss mice[5]. The data given in the Table show that all the cells tested have essentially the same sensitivity to free amanitin, whereas the macrophages are many times more responsive to AMA-BSA than the other cells. The sensitivity of neoplastic cells to AMA-BSA was not higher than that of non-neoplastic cells. This result, in agreement with RYSER's observations[8], throws some doubt upon the belief in a specific high uptake of proteins by tumour cells[9,10].

The present results, as well as previous data[5], indicate that conjugation with albumin gives to amanitin a selective toxicity for macrophages (= histiocytes) and suggest a possible approach to the therapy of pathological proliferations of these cells.

It is indeed possible that besides amanitin other cytotoxic compounds covalently bound to albumin are released in active form after penetration of the conjugate into the cells. If these compounds selectively damage the cells in proliferation, the resulting conjugates should kill proliferating histiocytes at concentrations ineffective for the non-dividing histiocytes as well as for dividing non-histiocytic cells, which display a low degree of protein uptake.

Such conjugates could be useful in the therapy of those reticulosarcomas which are composed by histiocytes[11,12] and in the treatment of histiocytic reticuloses[13].

It is worth noting that: 1. normal macrophages and polymorphonuclear leukocytes, which are also highly pinocytosing cells, do not proliferate[14,15]; 2. promonocytes and mielocytes which are the dividing precursors of these cells have a very low pinocytic activity[14,16]; 3. neoplastic proliferating histiocytes were found to be highly phagocytic[11-13,17].

In our future experiments we shall try to determine which compounds would kill neoplastic proliferating histiocytes in vitro at concentrations ineffective for non-dividing histiocytes. Then we shall attempt to conjugate such substances to albumin to test whether they are

Toxicity of AMA-BSA and α-amanitin for different cells

	AMA-BSA CPE_{25}	CPE_{100}	α-amanitin CPE_{25}	CPE_{100}
Macrophages	2 (0.05)	30 (0.8)	2.5	10
HeLa	>250 (6.7)		3	10
KB	>250 (6.7)		5	>10
HEp-2	>250 (6.7)		10	>10
RTC	150 (4.2)	>250 (6.7)	5	>10
MDBK	>250 (6.7)		10	>10
MKS-Bu100	250 (6.7)	>250 (6.7)	5	>10
HEF-SV	125 (3.4)	>250 (6.7)	2.5	>10
HEF	62 (1.7)	>250 (6.7)	5	>10
BHK	250 (6.7)	>250 (6.7)	2.5	10
VERO	>250 (6.7)		10	>10
CV-1	250 (6.7)	>250 (6.7)	—	—
MEF	>250 (6.7)		—	—

CPE_{25} and CPE_{100} correspond to the concentrations (μg/ml) of AMA-BSA or α-amanitin killing 25% or 100% of the cells. The amount of amanitin contained in AMA-BSA (μg) is shown in parentheses. HeLa, KB, HEp-2, neoplastic cells from human carcinomata; RTC, neoplastic cells from a methylcholanthrene-induced sarcoma in Fisher rats; MDBK, Madin-Darby bovine kidney cell line[19]; MKS-Bu 100, mouse embryo fibroblasts transformed by simian virus 40[20]; HEF-SV, human embryo fibroblasts tranformed by simian virus 40; HEF, normal human embryo fibroblasts; BHK, baby hamster kidney cells[21]; VERO, CV-1, green monkey kidney cells[22]; MEF, primary mouse embryo fibroblasts.

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released in active form after the penetration of conjugates into histiocytes[23].

Note added in proof: While this paper was in the press it came to our knowledge an article by TROUET et al., (Nature new Biol., *239*, 110, 1972) in which similar concepts were exposed and the effect of a complex daunorubicin-DNA on mouse L 1210 leukemia was tested with encouraging results.

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Riassunto. Viene descritta la particolare sensibilità dei macrofagi al coniugato amanitina-albumina. È inoltre prospettata e discussa la possibile attività antineoplastica di coniugati dell'albumina con sostanze inibenti la mitosi o la sintesi del DNA.

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Induction of in vitro Maturation in Oocytes of *Triturus* (Amphibia Urodela)

In the last few years, numerous studies have been conducted on the induction of in vitro oocyte maturation by progesterone in the anuran amphibians[1-4], while analogous research on the urodele has not, for the most part, produced satisfactory results[1].

Recently, it has been made possible to induce in vitro oocyte maturation by progesterone in the urodele *Triturus viridescens*[5,6]. The urodeles represent the animal group in which the morphology and the structure of the lampbrush chromosomes have been studied most extensively[7-9]. For this reason, we thought it would be interesting to verify the possibility of inducing in vitro oocyte maturation in other species of urodeles, with the particular aim of investigating the morphological changes of the lampbrush chromosomes during the maturing period.

Material and methods. The study was conducted on the ovarian oocytes of *Triturus cristatus carnifex* (Laurenti 1768) and of *Triturus vulgaris meridionalis* (Boulenger 1882). The morphology of the lampbrush chromosomes in these species is well known[8,10]. The specimens used came from the outskirts of Pisa and Naples. A total of 9 experiments, using 8 females of *T. c. carnifex*, and of 6 experiments, using 5 females of *T. v. meridionalis*, were performed in a period from December to April.

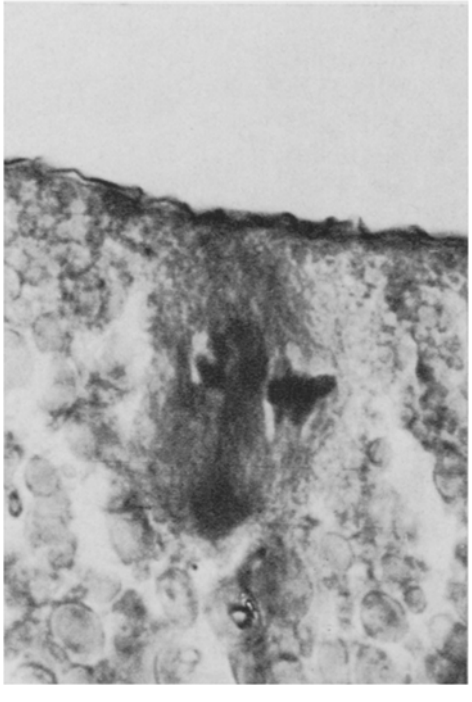

Fig. 1. First meiotic spindle in an oocyte of *T. c. carnifex*. 3 h from the time of incubation in progesterone. ×1520.

The females were pretreated with a gonadotrophic hormone ('Pregnyl', Organon). Each female received 3 injections, on alternate days, of 100 units each for *T. v. meridionalis* and of 200 units each for *T. c. carnifex*. One or both of the ovaries were then removed, and the larger oocytes were isolated by dissection in Ringer's solution for amphibians. The diameters of these oocytes measured between 1.5 mm and 1.75 mm in *T. c. carnifex*, and between 0.96 mm and 1.4 mm in *T. v. meridionalis*. Some of the oocytes were left in Ringer's and used as a control (67 oocytes for *T. c. carnifex* and 26 for *T. v. meridionalis*), and some were incubated for 1 h in Ringer's solution containing progesterone (Schering) at a concentration of 10 μg/ml, and then once again transferred to Ringer's (173 oocytes for *T. c. carnifex* and 49 for *T. v. meridionalis*).

The maturation process could be observed in the intact oocytes since the germinal vesicle, almost central in the immature oocytes, migrates toward the animal pole during maturation and becomes visible from the outside; at the end of the maturation, a small light area near the animal pole indicates the zone of formation of the second meiotic spindle and of expulsion of the first polar body. The germinal vesicle breakdown was also ascertained by dissection of the oocytes.

At various times, from the beginning of the period of incubation, some of the oocytes were fixed in Bouin's or in Goldsmith's solution and used for histological preparations; preparations of lampbrush chromosomes were made from other oocytes[11]. The control oocytes were processed analogously.

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