Negative Results of Attempts to Prolong the Effect of Phospholipids (PL) and Cholesterol (Chol) on the Formation of Tumours Induced by 3-Methylcholanthrene (MeC) *

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(Z. Naturforsch. **25 b**, 229—230 [1970]; eingeg. am 18. September 1969)

It was formerly demonstrated that PL retard and Chol accelerates the formation of tumours induced by MeC [1, 2]. This retardation respectively acceleration, however, never surpassed a period of somewhat more than four weeks. The question, then, arose why PL are only capable to retard temporarily and not to prevent, i. e. retard permanently, the formation of tumours. It was presumed that the administration of an excess of PL could favourably influence the time of retardation, namely by impeding the liberation of MeC from its molecular associations with PL through the interaction of Chol occurring everywhere in the organism:

$$\text{PL-MeC} + \text{Chol} \rightarrow \text{PL-Chol} + \text{MeC}.$$

A series of attempts have, therefore, been undertaken in order to increase respectively decrease the PL/Chol-ratio in the organism by the administration of excessive quantities of PL respectively Chol. The obtained results were all negative for neither the retardation by excess of PL, nor the acceleration by excess of Chol of the formation of tumours could be prolonged for more than four weeks.

Method and Results

Eleven groups of twenty Swiss mice each were formed.

The controls (Group I) were injected subcutaneously in one single dose with 0.5 mg MeC in 0.2 ml trioctanoin (Eastman-Kodak). Group II received 0.5 mg MeC plus 50 mg of "Asolectin" ** in 0.2 ml trioctanoin. This same injection was applied in the mice of Groups III, IV, V and VI to which, however, additional quantities of PL were given, namely: to Group III, already 35 days before the application of MeC, a 1% Asolectin-emulsion as the drinking water which corresponds with a daily dose of about 1.5 g PL/kg bodyweight; to Group IV: starting 15 days before the MeC-injection, three times weekly 0.1 ml of a 10% emulsion of Asolectin in saline 0.9% subcutaneously, corresponding with a dose of 1 g PL/week/kg bodyweight; to Group V: initiating 15 days before the MeC-dosage, equally three times weekly 0.1 ml of the same Asolectin-emulsion, intraperitoneally; and to Group VI: 0.1 ml of a 2% phosphatidylcholine-emulsion three times weekly in the tail-vein, corresponding with 200 mg PL/week/kg bodyweight.

The animals of Group VII were injected subcutaneously with a single dose of 0.5 mg MeC plus 20 mg Chol dissolved in 0.2 ml trioctanion, whereas those of Group VIII received, in addition to that injection and starting already 35 days before the MeC-administration, 4 g Chol dissolved in 80 ml babassu-fat *** per kg of ration which corresponds with a dose of about 1 g Chol/day/kg bodyweight. The additional quantities of Chol for Groups IX and X were administered subcutaneous respectively intraperitoneally three times weekly in doses of 0.1 ml of a 5% Chol-solution in babassu-fat slightly warmed at 40 °C to prevent Chol-crystallisation. This dose corresponds with about 500 mg Chol/week/kg bodyweight. Group XI, finally, received the additional dose of Chol intravenously in the form of an O/W-emulsion, containing 0.8% Chol and stabilized with sodium stearate. This emulsion was introduced three times weekly in a dose of 0.1 ml which corresponds with 75 – 100 mg Chol/week/kg bodyweight.

The appearance of tumours was controlled twice weekly from the 3rd to the 7th week and, thereafter, three times a week. The results are summarized in the Table 1, in which the three figures a/b/c represent: (a) the total number of living mice, (b) the number of living tumour-bearing mice, and (c) the number of mice died from cancer.

Discussion

It is seen from the figures in the Table 1 that in the twelfth week after MeC-injection, already half of the control mice had acquired cancer, whereas only 15% of Group II, about 36% of Group III, 22% of Group IV, and 28% of Group V beared tumours. In the Chol-groups, on the contrary, these percentages amount to not less than 89 for Group VII, 90 for Group VIII, 95 for Group IX, and 94 for Group X.

Thus, with the exception of the mice which have received either PL or Chol intravenously, it can be positively stated that PL retard the appearance of tumours, whereas Chol showed a pronounced accelerating activity. The same is true for the rate of mortality. Thirty weeks after the MeC-injection, namely, one animal of the control-group, three of Group II, one of Group III and two of Group IV escaped tumour-formation and survived, whereas already 16 weeks after MeC-administration, all mice of the Chol-groups have died from cancer, excepted those of Group VII which, however, were all tumour-bearing and, hence, condemned to death. The mice of Groups IV, V, IX and X

* Supported by a grant of the Brazilian National Research Council (Conselho Nacional de Pesquisas), Rio de Janeiro.

[1] R. F. A. ALTMAN, O. PUGACHIOV, I. BALLINI-KERR, and D. J. DA SILVA, Z. Naturforsch. **23 b**, 1277 [1968].

[2] R. F. A. ALTMAN, O. PUGACHIOV, I. BALLINI-KERR, and L. L. S. PINTO, Arch. Geschwulstforsch. **31**, 133 [1968].

** This granulated soybean phosphatide was gently put at our disposal by Mr. J. EICHBERG, President of American Lecithin Cy., Atlanta, USA.

*** Babassu-fat was gently put at our disposal by Dr. MOACYR SILVA, Technical Director of "Carioca Industrial, S.A.", Rio de Janeiro.

Table 1. Appearance of tumours after subcutaneous MeC-administration.

Group \ Weeks	7 a/b/c	10 a/b/c	12 a/b/c	16 a/b/c	25 a/b/c	30 a/b/c
I (Contr.)	18/4/0	18/7/0	18/9/0	14/10/4	3/2/15	1/0/17
II (PL, once only)	20/2/0	20/2/0	20/3/0	19/10/1	3/0/17	3/0/17
III (PL, oral)	14/0/0	14/4/0	14/5/0	14/10/0	2/1/13	1/0/14
IV (PL, s. c.)	18/1/0	18/2/0	18/4/0	18/11/0	4/2/14	2/0/16
V (PL, i. p.)	18/2/0	18/3/0	18/5/0	14/12/4	1/1/17	0/0/18
VI (PL, i. v.)	9/6/0	8/3/1	8/5/1	4/4/4	0/0/8	—
VII (Chol, once)	19/12/0	19/17/0	18/16/1	8/8/11	0/0/18	—
VIII (Chol, oral)	20/7/0	20/15/0	20/18/0	0/0/20	—	—
IX (Chol, s. c.)	19/7/0	19/15/0	19/18/0	0/0/19	—	—
X (Chol, i. p.)	16/7/0	16/13/0	15/14/1	0/0/16	—	—
XI (Chol, i. v.)	2/0/0	suspended				

must have suffered from the received subcutaneous and intraperitoneal injections for a large excess of unabsorbed PL-emulsion, respectively oil-solutions of Chol, was constantly found either subcutaneously or in the abdominal cavity.

A high mortality was observed in the intravenously injected mice, probably as a consequence of the imperfect condition of the introduced emulsions due to lack of an adequate stabilizer which, in the meantime, has been found in Pluronic F-68, a non-ionic, water-soluble polyoxyethyleneoxypropylene polymer produced by Wyandotte Chemical Corp., Wyandotte, USA [3].

It seems worth while to repeat the introduction of either PL or Chol directly in the bloodstream for the excess of these lipids when administered by other ways has not shown the slightest effect, probably due to metabolic modifications of their molecular structures. However it may be, it must be concluded from the above that the activity of PL and Chol is only perceptible when these compounds find themselves in direct molecular contact with the carcinogen. This confirms the assumed existence of molecular associations of PL and MeC from which MeC can be released by Chol, due to the higher affinity of the latter to PL (see the equation given in the Introduction). As was already explained before [1], PL-MeC associations are too large in size for occupying open spaces in the cell-membranes (cf. ALTMAN [4, 5]).

Experiments are now in progress to verify the activity of a large excess of PL and Chol introduced *together* with the carcinogenic hydrocarbon. At the same time, the influence of an excess of these lipids administered intravenously in the form of perfect emulsions is studied.

Thanks are due to V. C. DO AMARAL for his invaluable technical assistance.

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