REPORT

Effects of Ecomer on Immunological System in Mice

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REPORT

Effects of Ecomer on immunological system in mice. Part 1.

Material and methods.

Studies on the effect of Ecomer have been performed in 2-month old,male inbred Balb/c mice. Mice have been of local laboratory breed, weighing 20 g each.

Each mouse received Ecomer in dose of 12,5 mg/mouse/24 hrs, or water- during 7 days. On the day 8-th mice were subjected to narcose by means of chloral hydrate, bled from the retroorbital plexus and sacrificed. Spleens have been dissected under sterile conditions and spleen cell suspensions have been prepared .

The tests performed in the study:

1/ Counting of granulocytes and lymphocytes in heparinized peripheral blood.

2/Measurements of metabolic activity of blood granulocytes using chemiluminescence stimulated by Zymosan, according to the method described by Easmon & Cole (1) in own modification (2). Chemiluminescent activity was measured in scintillation counter (RackBeta 1218,LKB Wallac, Sweden) and expressed as *cpm* per 1000 granulocytes.

In addition to above, *the following complementary tests* have been performed:

1/ Local reaction graft- versus host (test LIA, lymphocyte-induced angiogenesis) according to Sidky and Auerbach(3). Briefly, multiple samples of splenocytes suspension (each sample five hundred thousands cells in 0,05 ml of Parker fluid), cleaned from erythrocytes by centrifugation on Histopaque 1077 gradient, were grafted intradermally into anaesthetised (Balb/c x C3H)F1 6-weeks old male mice. Number of newly formed blood

vessels has been calculated in dissection microscope after 3 days of implantation, using criteria described by authors of the method.

2/ Test of angiogenesis induced by cells isolated from mice cancer – Balb/c sarcoma L1.

Sarcoma cells were kindly delivered by dr.Henryk Skurzak from Warsaw's Oncology Center and then passaged through several generations of locally bred Balb/c mice.

Two hundred thousands sarcoma cells have been implanted (while suspended in 0,05 ml of Parker solution} into regionally shaved, narcotised Balb/c mice and angiogenesis estimated quantitatively after 3 days of implantation, on the inner skin surface, as described previously (4).

Results.

Results of the performed studies are presented in Table 1. Both number of circulating granulocytes ($p<0,001$) and their chemiluminescence activity,when measured in a scintilation counter increased significantly($p<0,05$) after Ecomer treatment.

Results of complementary additional studies are shown in tables 2 and 3. Treatment with Ecomer resulted in an increase of activity of splenic lymphocytes in a local graft-versus-host reaction (table 2).

Conclusions

Results of the performed studies indicate that Ecomer treatment induce an increase in cell-mediated nonspecific immunological defence {mainly the number and acyivity of granulocytes which constitute the first line of immunological defence). Ecomer treatment results also in an increase of specific immunological defence {the response of splenic lymphocytes to allogeneic histocompatibility antigens in the graft-versus-host reaction}.

Ecomer seems therefore a good preparation for patients with decreased cell-dependent immunological defence in the course of different diseases, in particular neoplasmatic diseases.

The application of Ecomer in cancer disease and its beneficial effect is indicated by its inhibitory effect on tumour angiogenesis. Anti-angiogenic effects of substances isolated from shark has been previously described by Sills et al.(5). These observations have been also confirmed by our own investigations on human cancer (unpublished own results).

Literature :

1. Easmon C.S.F., Cole P.J., Williams A.J., Hastings M. „The measurement of opsonic and phagocytic function by Luminol-dependent chemiluminescence." Immunology, 41, 67-74, 1980.
2. M.Filewska, E.Skopińska-Różewska, E.Sommer. "Wpływ oleju z nasion wiesiołka (EPO) na liczbę i aktywność chemiluminescencyjną granulocytów obojętnochłonnych krwi królików i myszy". Biologiczne Monitorowanie Skażenia Środowiska. Wpływ ksenobiotyków na układ odpornościowy. Pod red. A.K.Siwickiego, wyd.IRS, Olsztyn, 147-153, 1997.
3. Y.A.Sidky, R.Auerbach. „Lymphocyte-induced angiogenesis a quantitive and sensitive assay of the graft-vs-host reaction". J.Exp. med., 141, 1084-1100, 1975.
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5. A.K.Sills, J.I.Williams. „Squalamine inhibits angiogenesis and solid tumor growth in vivo and perturbs embryonic vasculature". Cancer Research 58, 2784-2792, 1998.

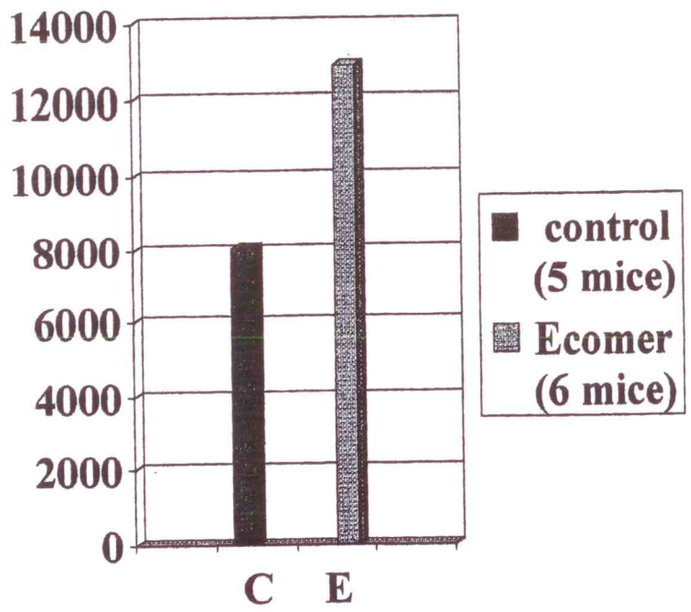
Table 1

The influence of Ecomer administered orally to balb/c mice 12,5mg/mouse/day for 7 days on number and activity of peripheral blood granulocytes evaluated by chemiluminescence test stimulated with zymosan *.

	Number of mice	Number of granulocytes in mm^3 ±SE	Statistical significance of difference	Chemiluminescence activity (CImax) ± SE	Statistical significance of difference
Control group (water)	5	552 ± 40		8030 ± 1401	
Experimental group (Ecomer)	6	873 ± 26	p<0,001	12881 ± 1751	p<0,001

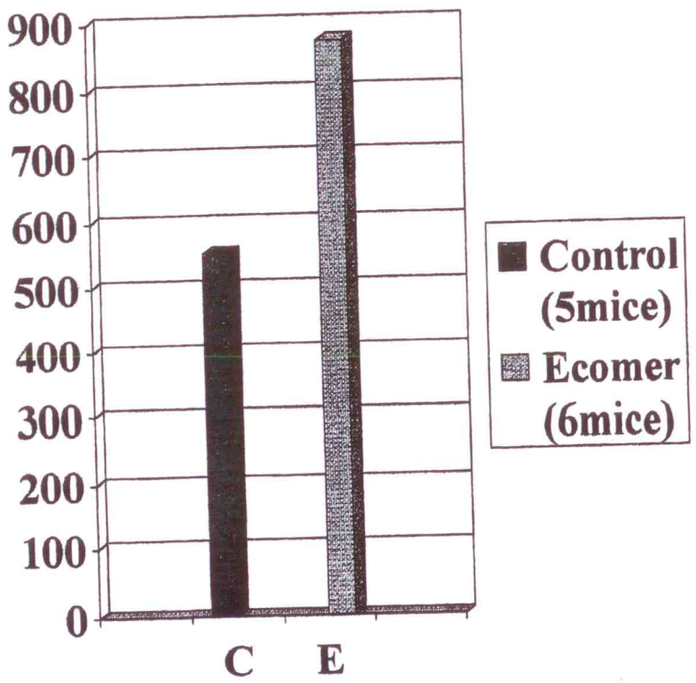
*** Mean number of lymphocytes/mm^3 of blood was 2608 ± 119 in group fed with water and 2508 in group fed with Ecomer (non statistical significance).**

The effect of Ecomer on chemiluminescent activity of blood granulocytes



- Mice were fed Ecomer for 7 days (12,5mg/mouse/day)
- Chemiluminescence was evaluated in whole blood (zymosan,luminol,)
- (Scintilation counter)
- Results expressed as mean cpm/1000 granulocytes
- $p<0,05$

The effect of Ecomer on granulocytes number in the peripheral blood of mice



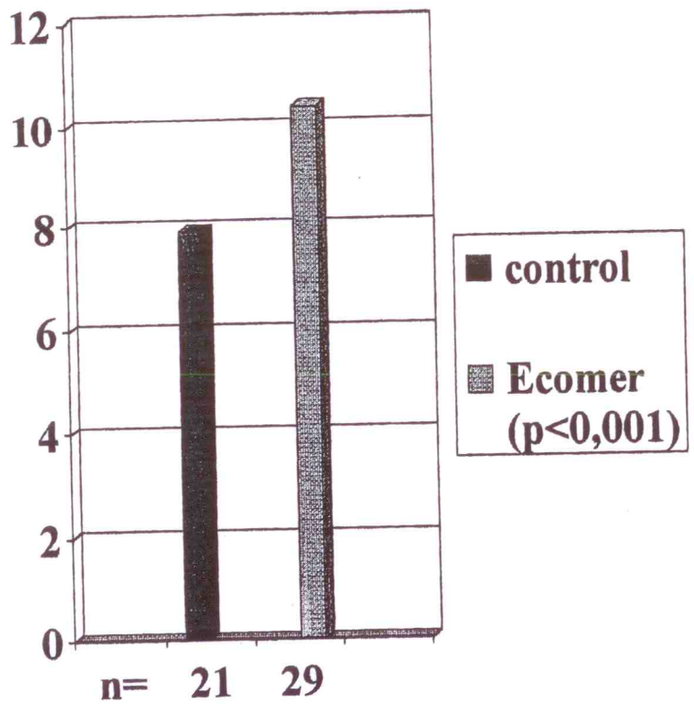
- Mice were fed Ecomer for 7 days (12,5mg/mouse/day)
- On the 8-th day mice were bled from retroorbital plexus
- $p<0,001$

Table2

The influence of Ecomer administered orally to Balb/c mice (12,5mg/mouse/day for 7 days) on activity of their spleen cells in local graft-versus host reaction. LIA test in (Balb/c x C3H) F1mice

	Number of tests	Mean number of newly formed blood vessels ± SE	Statistical significance of difference
Control group (water)	21	7,9 ± 0,4	
Experimental group (Ecomer)	29	10,4 ± 0,3	p<0,001

The effect of Ecomer on the graft-versus-host activity of spleen cells *(mean number of newly formed blood vessels)*



- Balb/c mice were fed Ecomer 12,5mg/mouse/day for 7 days.
- Their spleen cells were grafted intradermally to (Balb/cxC3H)F1 hybrids (LIA test).
- 3 days later neovascular response was evaluated in dissection microscope.

REPORT

Effect of Ecomer on immunological system in mice. Part II.

Material and methods.

Studies have been performed in Balb/c mice. These are locally breed mice - males of 20 g body weight, 2-months old.

Mice have been divided into two groups: one group received Ecomer in daily 0,05 ml dose of 12.5 mg/mouse/24hrs. Other group receives daily corresponding volume of water.

Following measurements have been undertaken in all experimental animals:

1. *Measurements of organ weight.* Spleens have been dissected, removed and weighted and splenocytes counted.
2. *Measurements of chemokinetic activity of splenocytes.*
   Chemokinetic activity of splenocytes has been estimated in the migration test in vitro according to modified Sandberg method (1, 2). Briefly, siliconised capillary tubes were filled with spleen cells suspension (2×10^7/ml of Parker medium) sealed with plasticine, and centrifuged for 5 min. at 300g. Then capillary tubes (at least 5 for one tested spleen in each experiment) were fixed on the glass plates and cells level have been marked.
   After 24 hours incubation (37^0C, 5% CO_2) the distance of migration will be measured in millimeters (mm) at a magnification of 6,5 x.
3. *Measurements of proliferation of splenocytes after PHA mitogen stimulaton.*
   The test is hased on measurements of labeled thymidine incorporation with radioactivity counting in the scintilation counter as described previously (3).

In addition to the above tests complementary examinations have been performed:

1. *Measurements of thymus weight and number of thymocytes.*
2. *Evaluation of the Ecomer effect on the production of antibodies.*
   Mice have been sensitised by peritoneal administration of 0,2 ml 10% sheep erythrocytes (SRBC).
   After 7 days all mice have been killed by sanguination.
   The titres of hemaglutinating antibodies in serum have been measured - as described previously (4,5).

Results.

1. *Measurements of organ weight of spleens and splenocytes counting.*
   No statistic significant deferences have been found between Ecomer treated and control group for the weight and cellularity of spleens.
2. *Chemokinetic activity of splenocytes.*
   Table 1 is showing the chemokinetic activity of splenocytes.
   Ecomer treatment resulted in statistic significant increase of this activity (mean migration distance being rnore than double as long as in the control group).
3. *Measurements of proliferation of splenocytes after PHA mitogen stimulation.*
   Results of these measurements are shown in table 3.
   A clear inhibition of the response to PHA stimulation has been noted for the 0.5 and 1.0 μg/ml concentratrons of PHA.
   No clear influence of Ecomer treatment has been found for the highest PHA concentration of 2 μg/ml.

Results of complementary tests.

1. No effect of Ecomer treatment has been found on the weight and cellularity of thymus.
2. Antibodies production.
   Effect of Ecomer treatment on the production of antibodies are summarised in table 2.
   Ecomer treatment resulted in a clear and statistically significant increase of antibodies production.
   Production of antibodies againist sheep's erythrocytes was found markedly increased ($p<0.001$).

Conclusions.

Ecomer treatment significantly increases humoral immunological defence and chemokinetic activity of spleen cells.

Ecomer treatment results in a decrease of the response to mitogen PHA stimulation. Decreased proliferation of splenocytes caused by Ecomer may depend on the increased percent of B lymphocytes observed in Ecomer treated mice. This

conclusion is further confirmed by the observation showing higher production of antibodies.

Ecomer presents a general pharmacologic characteristics indicating its pharmacologic applicability in the treatment of conditions with decreased humoral immunological defence.

References.

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3. P.Kamiński, E.Skopińska-Różewska, J.Bany, M.Wąsik, I.Sokolnicka, E.Barcz, E.Rogala, E.Sommer, W.Brajczewska-Fischer, M.Filewska, B.Bałan, P.Skopiński, L.Marianowski.: „The effect of salbutamol treatment on the cellular immunity of the offspring of pregnant mice: spleen cell activity". Drugs Exptl.Clin.Res, 2, 77-83, 1998.
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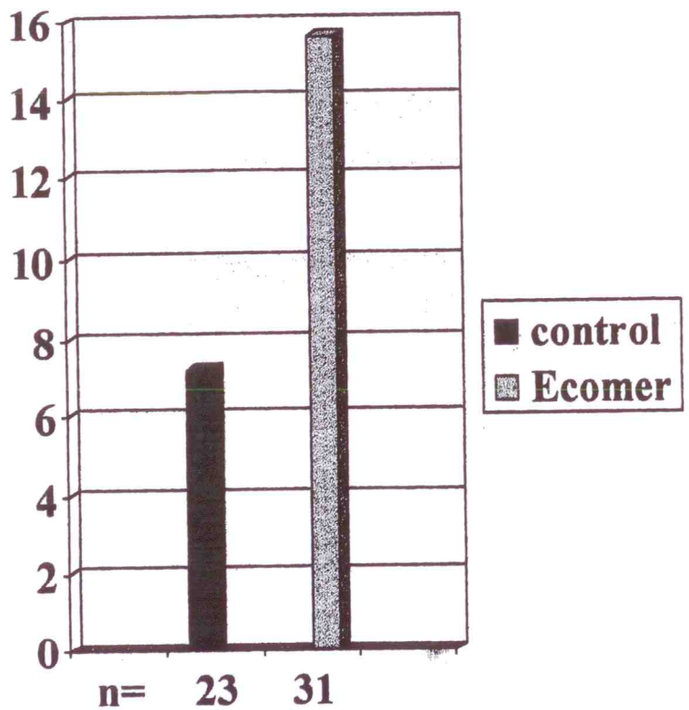
Table1

The influence of Ecomer administered orally 12,5mg/mouse/day for 7 days on Balb/c mice spleen cells migratory activity in vitro.

	Number of tests	Mean way of migration ± SE *	Statistical significance of difference
Control group (water)	23	7,2 ± 0,43	
Experimental group (Ecomer)	31	15,6 ± 0,91	p<0,001

*** one migratory unit = 0,18mm**

The effect of Ecomer on chemokinetic activity of spleen cells



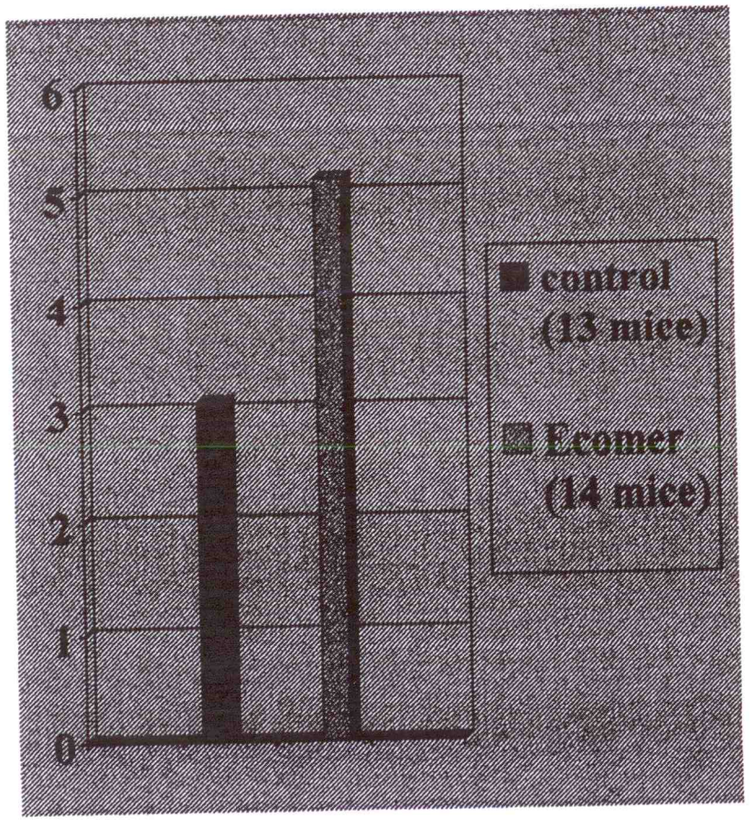
- Mice were fed Ecomer 12,5mg/mouse/day for 7 days.
- Spleen cell suspensions were evaluated for their migratory activity in 24hrs in vitro culture.
- 1 migratory unit=0,18mm.
- P<0,001

Table 2

The influence of Ecomer on antibodies against sheep erythrocytes production in Balb/c mice fed 12,5mg/mouse/day for 7 days.

	Number of mice	Mean log titer ± SE	Statistical significance of difference
Control group (water)	13	3,07 ± 0,32	
Experimental group (Ecomer)	14	5,14 ± 0,27	p<0,001

The effect of Ecomer on antibody production in mice



- Mice were fed Ecomer 12,5mg/mouse/day for 7 days.
- On the day 8-th mice were injected i.p. with 0,2 ml of 10% SRBC
- 7 days later mice were bled and sera tested for hemagglutinating activity (mean log titer)
- $p<0,001$

Table 3

The effect of Ecomer administered orally to Balb/c mice 12,5mg/mouse/day for 7 days on spleen cells activity in proliferation test stimulated PHA.

x cmp ± SE				
PHA concentration in culture	**0**	**0,5ug/ml**	**1ug/ml**	**2ug/ml**
Control group (water)	99 ± 8,1 n=11	2408 ± 131 n=11	4873 ± 289 n=12	5381 ± 643 n=11
Experimental group (Ecomer)	105 ± 17,5 n=11 n.s.	1735 ± 115*** n=12	3860 ± 203** n=12	3893 ± 414 n=11 n.s.

n.s. - non significant

** $p < 0,01$

***$p<0,001$