Effect of Ftorafur[1] on Acid Phosphatase Activity in the Liver of Mice with Transplanted Lymphatic Leukaemia

Our previous work with mice bearing transplanted lymphatic leukaemia showed release of acid phosphatase from liver lysosomes in the course of progressive infiltration of the liver by leukaemia, presumably due to labilization of lysosomal membranes of liver reticuloendothelium[2]. Such a phenomenon could be interpreted as a defence of the organism against malignant cells. Our present study was undertaken to assess the effect of an antineoplastic drug on this non-immunological resistance of the host against spread of neoplasia. We chose a newly synthesized fluoropyrimidine antimetabolite, Ftorafur N_1-(2'-furanidyl)-5-fluorouracil, which is an analogue of more commonly used 5-fluorouracil (5-FU).

Material and method. Adult female mice of A strain, 10 to 12 weeks old and weighing 18–20 g, were used in these experiments. The animals were divided into 4 groups of 15 mice each, and were treated according to schedules indicated in Table I. A lymphatic leukaemia, specific for A strain mice, described earlier by TONKOVIĆ and BORANIĆ[3], was used as the experimental model. 10 animals of each group were sacrificed on day 10 after inoculation, i.e., 9 days after inception of treatment with Ftorafur, and the remaining mice were observed until death. Ftorafur was administered via drinking water as a 0.05% solution in distilled water. Since each animal drank approximately 3 to 4 ml water daily (except when moribund a few hours before death), the calculated average dose of drug was 90 mg/kg/day. This dose was chosen on the basis of a preliminary experiment and represents $^1/_3$ of the LD_{50} value for a 9-day peroral administration of Ftorafur to mice.

The results of untreated leukaemic mice in this study (Group 2) were obtained in the same way as in our previous work[2]. 10 animals in each group were sacrificed on a predetermined day. The livers and spleens were immediately removed. Small pieces of both organs were excised and fixed in 10% buffered formalin, embedded in paraffin, sectioned, and stained with haemotoxylin-eosin. The remaining parts of the livers were weighed, minced with scissors and homogenized with Potter-Elvehjem glass-teflon homogenizer at 1000 rpm in ice cold 0.25 *M* sucrose containing 10^{-3} *M* EDTA (pH 7.0). Liver homogenate from 2 mice constituted a single pooled sample for enzyme assay and protein determination. The 5% tissue homogenates were assayed for total and unsedimentable acid phosphatase activities. Total enzyme activity was measured in the whole liver homogenates. The final incubation mixtures contained 0.1% Triton X-100 as well as suitable substrate and buffer. Addition of the detergent caused complete release of the enzyme activity[4]. The whole liver homogenates were centrifuged at $75{,}000 \times g$ for 1 h in a MSE High-Speed 25 centrifuge (8×50 ml head) to sediment particulate activity. The supernatant obtained was assayed in the presence of the same concentration of Triton X-100 as in determination of total activity and represents unsedimentable acid phosphatase activity. The ratio of unsedimentable to total activities was used as an index for in vivo release of acid phosphatase from lysosomes. Acid phosphatase (EC 3.1.3.2) was determined by the method of BERTHET and DE DUVE[5]. Units of enzyme activity are expressed as μmoles of inorganic phosphate (P) released/h/g

Table I. Treatment schedules

Group	10^6 leukaemic spleen cells i.v.	Ftorafur (90 mg/kg/day per os)
1	–	–
2	+	–
3	+	+
4	–	+

[1] N_1-(2'-furanidyl)-5-fluorouracil.
[2] O. CAREVIĆ, V. ŠVERKO and M. BORANIĆ, Eur. J. Cancer, *9*, 549 (1973).
[3] I. TONKOVIĆ and M. BORANIĆ, Yugoslav. physiol. pharmac. Acta, *9*, 97 (1973).
[4] R. WATTIAUX and C. DE DUVE, Biochem. J. *60*, 604 (1955).
[5] J. BERTHET, L. BERTHET, F. APPELMANS and C. DE DUVE, Biochem. J. *50*, 182 (1951).

Table II. Wet weight of liver, protein content, total and unsedimentable acid phosphatase activities in the whole liver homogenates of normal and leukaemic mice treated or not with Ftorafur (90 mg/kg) for 9 days

Group	Treatment	Liver weight (% of body weight)	p[a]	Protein (mg/g liver)	p[a]	Acid phosphatase			
						Total (μmole P/h/g of liver)	p[a]	Unsedimentable (% of total)	p[a]
1	Untreated-control	4.53 ± 0.82 (10)	–	198 ± 2.05 (5)	–	98.32 ± 4.21 (5)	–	29.75 ± 1.07 (5)	–
2	Leukaemic cells (10^6)	9.65 ± 2.21 (10)	<0.05	172 ± 6.42 (5)	<0.01	69.48 ± 5.17 (5)	<0.01	71.47 ± 3.12 (5)	<0.01
3	Leukaemic cells 10^6 + Ftorafur (90 mg/kg) for 9 days	5.18 ± 1.78 (10)	>0.1	190 + 4.37 (5)	>0.1	84.76 ± 4.09 (5)	<0.05	25.18 ± 1.43 (5)	<0.05
4	Frorafur (90 mg/kg) for 9 days	4.08 ± 1.03 (10)	>0.1	191 ± 3.51 (5)	>0.1	89.27 ± 3.96 (5)	>0.1	24.02 ± 2.16 (5)	<0.05

Values for protein concentration and enzyme activities are given as means ± standard errors of the means for 5 specimens, each of which consisted of 2 pooled livers. Values for liver weight represent means ± standard errors of the means for 10 animals. [a] p = level of significance of the difference between group 1 and groups 2, 3 or 4. Number of experiments in parentheses.

wet liver. Protein content was measured by the method of LOWRY et al.[6].

Results. 5 of the 15 mice inoculated with leukaemic spleen cells died spontaneously 8 to 10 days after inoculation and the 10 surviving animals were sacrificed on day 10 for biochemical studies. The livers and spleens of these animals were enlarged and showed almost complete obliteration of normal structures by leukaemia cells. 15 mice inoculated with 10^6 leukaemic spleen cells and treated for 9 days with Ftorafur (90 mg/kg) survived for 10 days. 10 animals from this group were sacrificed on day 10 after inoculation; very few leukaemia cells could be detected in the portal areas of their spleens and livers. The remaining 5 animals received 90 mg/kg Ftorafur until death which occurred between 40 and 48 days, with hemorrhage into the gut and brain, but without morphological evidence of leukaemia in livers and spleens. 15 mice were treated with Ftorafur (90 mg/kg) for 9 days. 10 animals of this group were sacrificed on day 9 and the remaining 5 animals received the same dose of Ftorafur until death, which occured between day 47 and 58, with hemorrhage into the gut or brain. The livers and spleens of sacrificed mice were histologically normal (Table II).

The significance of the difference between various parameters are calculeted by Student's t-test. Liver weight, as percent of body weight, significantly ($P < 0.05$) increased in leukaemic mice (Group 2). This was paralleled by a decrease in protein content ($P < 0.01$) and in total acid phosphatase activity ($P < 0.01$) and by enhancement of unsedimentable activity ($P < 0.01$) in whole liver homogenates. Similar changes were observed in our previous study on 8 pooled livers[2]. In leukaemic and non-leukaemic mice treated with Ftorafur (Groups 3 and 4) liver weight and protein content remained essentially normal. On the other hand, total acid phosphatase activity was evidently higher ($P < 0.01$) in the livers of leukaemic animals treated with Ftorafur (Group 3) than in leukaemic mice without the drug (Group 2), but still did not reach the control value (Group 1). However, no significant difference in this parameter was established between leukaemic and non-leukaemic mice after treatment with Ftorafur (Groups 3 and 4). Unsedimentable activity was somewhat lower ($P < 0.05$) both in leukaemic and in normal animals treated with Ftorafur (Groups 3 and 4) as compared with controls (Group 1) but related to leukaemic mice (Group 2) the difference was almost 3-fold.

Discussion. Prolonged survival of leukaemic mice treated with Ftorafur, and reduced infiltration of their spleens and liver by leukaemia cells indicate an antileukaemic effect of Ftorafur. The drug produced no toxicity until sacrifice of mice on day 10 post inoculation. Prolonged administration of Ftorafur caused haemopoietic toxicity and death. Further discussion will be based on the assumption that the activity of liver acid phosphatase, a lysosomal marker enzyme, actually reflected response of liver reticuloendothelium to the invasion of the liver by leukaemia cells[2]. Decreased unsedimentable acid phosphatase activity in leukaemic animals after treatment with Ftorafur may be explained by a) an inhibitory effect of Ftorafur on growth of leukaemic cells in the liver, b) by decreased fragility of lysosomes caused by Ftorafur in vivo or c) by both effects. The fact that unsedimentable acid phosphatase activity significantly decreased in normal mice after treatment with Ftorafur supports the idea that this drug indeed decreased the fragility of lysosomes. Many authors observed an increased number of lysosomes and release of their enzymes after treatment with antineoplastic drugs or X-rays. They considered this autophagic vacuole response to be a prerequisite for the action of drugs on tumors[7–9]. Our results indicate, in the contrary, stabilization of lysosomes by Ftorafur. We envisage that upon contact with malignant cells lysosomes of liver reticuloendothelium become labilized and release their enzymes into cytoplasm of reacting cells[2]. If lysosomal vesicles remained stable, when reticuloendothelium encountered malignant cells, they would be available for fusion with phagocytic vacuoles. In that case malignant cells could be digested and destroyed intravacuolarly. The present results are in favour of the idea that antileukaemic effect of Ftorafur would be assisted by its ability to stabilize lysosomes.

Zusammenfassung. Nachweis, dass Ftorafur, N_1-(2'-Furanidyl)-5-Fluorouracil das Überleben leukämischer Mäuse deutlich steigert und ausserdem als Lysosomen-Stabilisator wirken könnte, wobei die in vivo Stabilisierung der Lysosomen den antileukämischen Effekt dieses Zytostatikums unterstützen könnte.

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[6] O. H. LOWRY, N. J. ROSENBOURGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).
[7] D. BRANDES, E. ANTON and K. W. LAM, J. natn. Cancer Inst. *39*, 385 (1967).
[8] J. E. PARIS, D. BRANDES and E. ANTON, J. natn. Cancer Inst. *42*, 383 (1969).
[9] J. PARIS and D. BRANDES, Cancer Res. *31*, 392 (1971).

Cholesterol Oxidation by Rat Liver Preparations: Effect of Age

Cholesterol synthesis is significantly reduced in aging rats[1–3]. YAMAMOTO and YAMAMURA[4] confirmed the fact that 5- or 8-month-old rats convert less acetate to cholesterol than do 2-month-old rats. They also reported that the older rats excreted fewer acidic steroids in their bile and feces. We have compared the activity of the two hepatic enzyme systems involved in bile acid synthesis from cholesterol in young (2 month) and old (18 month) rats.

The rats used were males of the Wistar strain. The two enzyme systems assayed were the mitochondrial system described by WHITEHOUSE, STAPLE and GUREN[5] which is involved in the oxidation of the cholesterol side chain, the final step in bile acid synthesis[6]; and the microsomal 7α-hydroxylase, the first and rate-limiting step in bile acid synthesis from cholesterol[7].

The oxidation of [26-^{14}C] cholesterol to $^{14}CO_2$ was carried out according to published procedures[5,8]. Incubations were carried out in stoppered 125 ml Erlenmeyer flasks containing center wells. The incubation mixture consisted of 1 ml of mitochondrial preparation; 1 ml of a solution containing adenosine triphosphate