statistical significance of differences was assessed by the use of Student's t-test.

Mitochondria were incubated in the presence of malate and glutamate (2.5 mM each). The addition of ADP (0.5 mM) increased O_2 uptake by 68.9 ± 10.1% (paired comparison; $n = 22$; $p < 0.001$). In the presence of ADP, the respiratory rate averaged 19.1 ± 1.9 pmol of O_2 per min and per mg wet weight of cerebellum tissue ($n = 15$). In the presence of ADP, the addition of D-glucose (0.13 mM or more) further increased O_2 uptake by 22.8 ± 3.1% (paired comparison; $n = 59$; $p < 0.001$). The magnitude of the latter increase was little affected by the concentration of D-glucose in the 0.13–8.0 mM range, which is in large excess of the K_m for the hexose of mitochondrial brain hexokinase[6]. The relative magnitude of the hexose-induced increase in O_2 uptake was inversely related, however, to the ADP concentration (0.1 to 1.0 mM), decreasing ($p < 0.01$) from 35.2 ± 2.4% ($n = 3$) at low ADP concentration (0.1 mM) to 12.2 ± 4.9% ($n = 6$) in the presence of 1.0 mM ADP. When D-mannoheptulose (10.0 mM) was added to mitochondria already exposed to both ADP (0.5 mM) and D-glucose (0.13 mM), the rate of O_2 uptake was decreased by 15.3 ± 3.5% (paired comparison; $n = 3$; $p < 0.05$) and, hence, became close to that recorded prior to the introduction of D-glucose (fig.). Glucose 6-phosphate (3.0 mM) exerted an inhibitory effect comparable to that of D-mannoheptulose (data not shown). Moreover, when either mannoheptulose or D-glucose 6-phosphate were present in the medium together with ADP prior to the addition of D-glucose, the hexose failed to stimulate respiration (paired change: + 0.3 ± 1.5%; $n = 6$; $p > 0.8$). In the presence of ADP but absence of D-glucose, neither mannoheptulose nor glucose 6-phosphate inhibited O_2 uptake ($n = 6$).

The present results document that D-glucose increases state 3 respiration in brain mitochondria. In a prior study[9], an effect of glucose upon O_2 uptake by brain mitochondria had been observed after but not during ADP stimulation. In the present work, the effect of D-glucose appeared to be related to the capacity of the hexose to act as a substrate for mitochondria-bound hexokinase since it was suppressed by D-mannoheptulose or D-glucose 6-phosphate[6,10]. Incidentally, the fact that the response to D-glucose was inhibited by D-glucose 6-phosphate and modulated by the concentration of ADP strongly suggests that it indeed results from the stimulation of mitochondria rather than contaminating synaptosomes. Further work is required to assess such items as the concentration-relationship of the response to D-glucose; its stoichiometry relative to both ADP and hexose phosphorylation as well as substrate oxidation; its anomeric specificity; its possible simulation by other substrates (e.g. glycerol) susceptible to be phosphorylated by porin-bound kinases such as glycerol kinase[11], and its physiological relevance.

Meanwhile, our findings are compatible with the view that the phosphorylation of glucose as catalyzed by bound hexokinase may directly affect mitochondrial respiration through the consumption of ATP and concomitant generation of ADP[6].

Acknowledgments. This study was supported by a grant from the Belgian Foundation for Scientific Medical Research. The authors wish to thank G. Vandenbroeck for technical assistance and C. Demesmaeker for secretarial help.

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0014-4754/88/070610-02$1.50 + 0.20/0

Enhanced toxicity of the immunosuppressant ovalicin upon application to the skin

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Received 19 February 1988; accepted 10 March 1988

Summary. The non-myelotoxic immunosuppressive sesquiterpene ovalicin, of fungal origin, is much more toxic when applied to the skin of animals than when injected i.v., the LD-50 in guinea pigs being 0.2 in the first case and 7 mg/kg in the second. It elicits aphagia and adipsia. It is assumed that ovalicin effects are due to slow, tissue-specific, metabolic toxification.
Key words. Ovalicin; immunosuppression; toxicity; metabolic toxification; skin.

Screening fungal broths for cytostatic activity, using mouse mastocytoma P-815 cells, had already led to the finding of a number of interesting compounds like phomin (cytochalasin B), verrucarins and anguidine (see Stähelin[1]) when, in 1962, culture filtrates of the fungus *Pseudeurotium ovalis* Stolk were found to inhibit proliferation of mastocytoma cells. The metabolite responsible for this effect was then isolated[2,3] and turned out to be a sesquiterpene with two epoxy groups. The compound, which was given the name ovalicin, exhibited potent cytostatic activity against P-815 cells in vitro with an ID-50 below 1 ng/ml. Despite its comparatively low toxicity it did not inhibit the growth of P-815 tumor cells in mice, but reduced the spleen weight of the treated animals. For this and other reasons, the compound was tested for immunosuppression and turned out to considerably decrease the formation of hemagglutinins in mice immunized with sheep erythrocytes. Further evaluation showed ovalicin to be a quite effective immunosuppressant in a number of tests (skin transplantation, experimental allergic encephalomyelitis and others) without reducing the number of granulocytes in the blood of the treated mice and rats[4–7]. In other studies, a high in vitro potency of ovalicin as inhibitor of

lymphocyte stimulation was shown and the mechanism of action investigated at the biochemical level [8, 9].

In the course of chronic tests with rats we had observed that ovalicin was much more toxic when given in the feed than when administered by gavage. It was considered possible that contact of the drug with the skin or perhaps the oral or respiratory mucosa might be responsible for the increased toxicity. This hypothesis was then tested experimentally; the results of these earlier investigations are reported now because some recent observations [10] in rabbits treated with cyclosporin A (Sandimmun®), the successor of ovalicin [1], are reminiscent of some findings with ovalicin.

Methods. Clear aqueous solutions of ovalicin for i.v., i.p. and s.c. administration were prepared from a lyophilized lot by dissolving the drug powder in a small amount of dimethyl sulfoxide; then Tween 80 and, after mixing, water was added to obtain the desired concentration. Control animals received the solvent. The day of (first) administration of the compound is day 0.

Animals: Mice were females of the F-1-generation of Sandoz-inbred Swiss mice x DBA/2, weighing 18–22 g. Rats were females of the CFE-strain, bred and brought up in the animal farm of Sandoz Ltd., weighing 180–250 g. Guinea pigs were albinos of both sexes, weighing 250–350 g. Syrian hamsters were random bred and weighed 120–150 g. The animals were kept in plastic (macrolone) cages of size 2 (mice) or 3 (hamsters, rats, guinea pigs) and had free access to food pellets and water. Bedding in the cages was sawdust, about 60 g for size 2 cages and ca. 160 g for size 3 cages. 4 mice, 3 rats or 3 guinea pigs were housed in one cage.

Results. In order to imitate to some degree the situation when drug is added to the feed (drug powder mixed with ground food pellets), 20 mg of ovalicin powder per cage was mixed with the sawdust bedding of the animals and their body weight and mortality were recorded. Rats exhibited a decrease of body weight (12%) by day 6, but all animals survived. Weight loss in mice was more severe (37% on day 11) and all animals died (median survival time: 12.5 days). Guinea pigs reacted even more strikingly; they lost 25% of their body weight within 48 h and, after further body weight loss, all 3 animals succumbed before day 6. This body weight decrease was due to an almost complete cessation of food and water intake. Upon exposure to ovalicin, guinea pigs became highly irritable and quite often assumed a peculiar position: they stood for several minutes in a corner of the cage with the head lifted upwards and the lower jaw resting on the cage wall, and bared their teeth ('ovalicin position'). Smaller amounts of drug powder were also added to the bedding of guinea pigs, so that an LD-50 could be calculated; it was found to be 0.5 mg/kg for continuous exposure (4 days) or 1.5 mg/kg for an exposure time of 24 h, after which time the animals were placed in new cages without drug (table).

In order to quantitate skin uptake of ovalicin more satisfactorily, the compound was dissolved in ethanol and applied to the shaved skin of guinea pigs (1 ml/kg to about 7 cm^2 of skin). Animals were transferred to new cages and new bedding after each application. Guinea pigs so treated exhibited the same symptoms as those with ovalicin in the bedding (excitation, ovalicin position, aphagia and adipsia). LD-50 by the epicutaneous route was 0.2 mg/kg for a single administration and 0.1 mg/kg (total dose) for repeated (5 ×) daily application (i.e., 0.02 mg/kg/day). Mice and rats are less susceptible than guinea pigs to ovalicin applied to their skin. Intracutaneous injection of ovalicin proved as lethal to guinea pigs as application on the epidermis (table).

These results suggest that one factor for the increased toxicity of ovalicin, when it is taken up through the skin, may be a perhaps low, but continuous blood and tissue level over a period of 2 or more days. To test this hypothesis, ovalicin was administered to guinea pigs i.v. with varying schedules. The LD-50, when the drug was injected i.v. once as a bolus, was found to be 7 mg/kg (table); the animals died within 5 days. When i.v. injections were given daily for 4 days, already a dose of 0.1 mg/kg/day (total dose 0.4 mg/kg) was lethal for all 6 animals, median survival time being 7 days; this comparatively long survival time suggests that the LD-50 is only slightly below 0.1 mg/kg/day. When infused continuously into the jugular vein for 4 days with an infusion pump, ovalicin is also very toxic to guinea pigs; the lowest tested dose of 0.032 mg/kg/day (total dose 0.13 mg/kg) killed all 6 animals with a median survival time of 3.5 days. Repeated s.c. injection is somewhat less toxic than the i.v. route (table).

Approximate LD-50 values of ovalicin

Species	Route	Schedule/ exposure time	LD-50 mg/kg total dose
Mouse	i.v.	once	> 100
	i.p.	once	> 1000
	i.p.	daily, 5 ×	1250
	bedding	11 days	< 200
	epicut.	daily, 5 ×	> 50
Rat	i.v.	once	> 200
	i.p.	daily, 5 ×	> 600
	bedding	11 days	> 33
	epicut.	daily, 5 ×	> 50
Syrian hamster	bedding	6 days	> 100
Guinea pig	i.v.	once	7
	i.v.	daily, 4 ×	< 0.4
	i.v.	96 h contin.	< 0.13
	i.p.	once	5.5
	i.p.	daily, 5 ×	4
	s.c.	daily, 5 ×	1.5
	bedding	24 h	1.5
	bedding	4 days	0.5
	epicut.	once	0.2
	epicut.	daily, 5 ×	0.1
	intracut.	daily, 5 ×	0.1

LD-50 values for drug added to the bedding are calculated based on the assumption that all drug was incorporated by the animals.

In mice and, more clearly, in guinea pigs, prolongation of exposure to ovalicin (without augmenting total dose) thus increases the mortality, and this is (in guinea pigs) true for different application routes. Oral toxicities are not reported here; the LD-50 values for the oral route (by gavage, i.e., by stomach tube) were always higher than for the other routes; there was, however, no difference in immunosuppressive potency between i.p. and p.o. administration in mice [5].

Guinea pigs treated with ovalicin epicutaneously, 5 × 0.025 mg/kg/day, exhibited a reduced thrombocyte count (– 64%) on day 3, while the number of leucocytes in their blood was not affected (results not shown). No formal pathological or histological examination was performed in any of the above-mentioned experiments, but in a few treated guinea pigs the lungs were removed after death; they exhibited red spots on their surface and showed histologic signs of hemorrhage and desquamation of epithelia in the alveoli and bronchioles (R. Griffith, personal communication).

The 'ovalicin syndrome' (strange behavior, aphagia, adipsia) has not been observed in acute tests (single i.v. and p.o. administration) in mice, rats and rabbits nor in prolonged experiments (4 weeks oral treatment) in rats and dogs.

Discussion. Ovalicin is about 35 times more toxic (lethal) to guinea pigs when applied to the skin than when injected i.v. When the compound is injected i.v. in divided doses (i.e., once daily for 4 days), LD-50 is at least 17 times smaller than when it is given as a bolus. Similarly, upon continuous i.v.

infusion for 96 h, toxicity is again very high, LD-50 being more than 50 times lower than after a single injection. Blood and tissue levels can be assumed to run a similar course after epicutaneous administration and during continuous infusion of small doses. This similarity suggests that high toxicity is related to a pharmacokinetic feature, namely long persistence of drug in the blood and/or tissues. This in turn could indicate that some in vivo metabolic process of low capacity transforms ovalicin into a highly toxic compound, and that this process is more active in guinea pigs than in the other animals tested which are less susceptible to the drug. Higher drug doses given p.o., i.v. or i.p. may be eliminated before the slow metabolism has toxified enough of the compound to produce lethality. Apparently, the toxicity of the metabolite is cumulative. A similar situation was observed with the cyclic peptide chlamydocin, which also contains an epoxy group; the LD-50 of this cytostatic compound is 23 times higher when it is injected i.v. as a bolus than when it is infused i.v. over 96 h[11]. Unfortunately, no pharmacokinetic data are available for ovalicin.

The symptoms elicited by cutaneous ovalicin administration suggest that toxicity is mainly due to an effect on the central nervous system. Whether lethality is also brought about by a central action is not clear. Since tranquilizing the drug-treated animals with a neuroleptic (thioridazine) or administration of fluid did not reduce lethality (results not shown), neither the excitation nor the adipsia provoked by ovalicin seems to be the cause of death.

The question arises, where metabolic toxification of ovalicin takes place. Is it in the liver? The comparatively high therapeutic index for the immunosuppressive activity of ovalicin suggests that the lymphatic system may be a tissue where toxification takes place. This would be compatible with the high in vitro toxicity of the compound for lymphocytes. P-815 mastocytoma cells, for which in contrast to other cell lines ovalicin is toxic too, may also convert the drug into a toxic metabolite. Is there perhaps even some toxification in parts of the central nervous system, eliciting thus the ovalicin syndrome? Such questions cannot be answered yet, but studies to elucidate these problems may lead not only to a better understanding of the effects of ovalicin but also to new ways to arrive at lymphocyte-specific, immunosuppressive compounds.

Acknowledgment. The skillful technical assistance of Armin Trippmacher and Barbara Burckhardt is gratefully acknowledged.

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0014-4754/88/070611-03$1.50 + 0.20/0

Effect of high doses of somatostatin on adenylate cyclase activity in peripheral mononuclear leukocytes from normal subjects and from acute leukemia patients

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Received 10 November 1987; accepted 9 March 1988

Summary. In normal lymphocytes somatostatin non-competitively inhibited basal (ID_{50} 5×10^{-4} M) and isoproterenol- and forskolin-stimulated adenylate cyclase activity (Ac). In acute leukemia blasts, non-responsive to isoproterenol, forskolin, which activates the catalytic subunit, stimulated and somatostatin inhibited Ac, thus indicating the leukemic enzyme, though defective, retains the inhibitory pathway and catalyst function.
Key words. Adenylate cyclase; somatostatin; forskolin; isoproterenol; lymphocytes; leukemic cells.

Somatostatin, a peptide originally isolated from the hypothalamus, as GH release inhibiting factor, has subsequently been located in many different tissues where it influences a wide variety of cellular processes[1]. Receptors for somatostatin have also been found in human mononuclear leukocytes[2] and evidence is accumulating that this peptide may modulate some lymphocyte functions. In vitro experiments have demonstrated that somatostatin at a concentration of 10^{-7} M stimulates lymphocyte proliferation and abolishes the antiproliferative effect of rat hypothalamic extracts[3], whereas at lower concentrations it clearly inhibits both spontaneous and mitogen-stimulated lymphocyte proliferation, as well as immunoglobulin synthesis induced by concanavalin A[3-6]. The mechanisms by which somatostatin affects lymphocyte functions are still unknown. In many tissues, however, the effects of the peptide have been related to its ability to inhibit cyclic AMP (cAMP) production[7-11]. It seemed therefore to be interesting to investigate the possible influence of different doses of somatostatin on adenylate cyclase activity a) in peripheral mononuclear leukocytes from normal subjects, and b) in human leukemic leukocytes, which are known to have defective adenylate cyclase unresponsive to various agents[12-16].

This paper presents evidence that in both normal and leukemic leukocytes somatostatin does not influence adenylate cyclase activity at physiological concentrations, nor at the pharmacological concentrations usually employed, but at higher doses of the peptide enzyme activity is inhibited.

Materials and methods. Eight healthy subjects and 9 untreated acute leukemia (3 lymphoblastic, ALL; 6 non-lymphoblastic, ANLL) patients with a high leukocyte count volunteered for this study. Peripheral blood was drawn with heparin as anticoagulant. As previously described[15], normal mononuclear leukocytes were separated by centrifuging the blood on Ficoll-Hypaque density gradients, whereas leukemic cells were isolated by spontaneous sedimentation at