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SHORT COMMUNICATIONS

Effect of oxamniquine therapy on kynurenine metabolism in liver homogenates of normal and *S. mansoni* infected mice

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Oxamniquine is a non-antimonial antischistosomal drug mainly effective against *Schistosoma mansoni*. Oxamniquine (6-hydroxymethyl-2-isopropyl aminomethyl 7-nitro. 1,2,3,4 tetrahydroquinoline) is a promising single-dose agent for the treatment of *Schistosoma mansoni* infection in man [1]. It is more effective than hycanthone, lucanthone and niridazole [2].

It was previously shown that the antimonial drug (tartar emetic), which is used extensively for the treatment of bilharziasis, inhibited both kynureninase and kynurenine transaminase in normal mouse liver [3] and kidney homogenates [4]. This inhibition resulted in a change in the relative concentrations of the different metabolites in the kynurenine pathway of tryptophan metabolism. Since many of these metabolites are known bladder carcinogens [5–7], this effect might be related to the development of bladder tumours in bilharzial patients treated with tartar emetic [8]. The antimonial content in this drug was held to be responsible for the disorder encountered in kynurenine metabolism. Therefore, the effect of oxamniquine on kynurenine metabolism and its conversion to kynurenic acid and anthranilic acid through the B_6-dependent kynurenine aminotransferase and kynurenine hydrolase enzymes, respectively, was studied in this work. In this follow up study kynurenine metabolism was investigated in tissue liver homogenates of both control and infected mice treated with oxamniquine.

MATERIALS AND METHODS

Animals. Adult albino mice, 2 months old and 15 g weight, were used. They were divided into four groups: group I included neither treated nor infected animals and served as controls; group II included uninfected but treated animals and served to study the effect of oxamniquine; group III included *S. mansoni*-infected animals; group IV included infected animals treated with oxamniquine. Mice in groups III and IV were infected by the paddling technique [9] with 100 *S. mansoni* cercariae. Fifty days from infection oxamniquine was given in an oral single dose (50 mg/kg) to animals of groups II and IV. Mice were then killed at 10, 40 and 70 days from treatment, i.e. 60, 90 and 120 days from infection, respectively.

Preparation of homogenates and incubation medium were carried out as previously reported [8].

Quantitative estimation of metabolites. Anthranilic acid was determined by the method of Mason and Berg [10]. The quantitative determination of kynurenic acid was performed by the method of Satoh and Price [11].

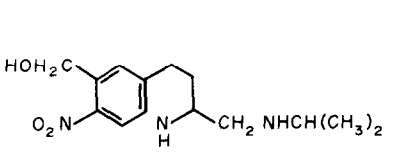
RESULTS

The activities of kynurenine aminotransferase and kynurenine hydrolase are indicated by the amounts of kynurenic acid and anthranilic acid produced in μmoles/g liver, respectively.

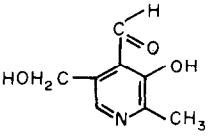
Table 1. The effect of duration on kynurenine metabolism in liver homogenates of normal and infected mice treated with oxamniquine

Group	No. of mice	Mean value (± S.E.) Kynurenic acid	Anthranilic acid
Group I: Controls			
(a) 4-months-old	7	3.90	1.63
(b) 5-months-old	8	4.64	1.42
(c) 6-months-old	8	4.83	2.00
Group II: Treated mice			
(a) 10 days after treatment	7	4.08 (0.38)	0.54* (0.07)
(b) 40 days after treatment	10	5.13 (0.53)	0.49* (0.12)
(c) 70 days after treatment	13	2.76* (0.26)	0.41* (0.06)
Group III: Infected mice			
(a) 60 days after infection	8	2.78 (0.55)	0.65* (0.11)
(b) 90 days after infection	13	4.28 (0.29)	1.17 (0.19)
(c) 120 days after infection	7	3.61* (0.42)	1.37 (0.42)
Group IV: Infected treated mice			
(a) 60 days after infection	9	3.41 (0.67)	0.56* (0.14)
(b) 90 days after infection	9	3.35 (0.53)	0.95 (0.23)
(c) 120 days after infection	9	2.61* (0.23)	0.79* (0.13)

* Statistically significant from the corresponding control value.



Oxamniquine



Pyridoxal

Fig. 1.

The statistical evaluation of these results was done by using the standard *t*-test [12] and are summarized in Table 1. A comparison yielding a probability value of less than 0.05 was considered significant. From these results it was found that, in uninfected animals given oxamniquine (Group II, Table 1) a normal amount of kynurenic acid was produced up to 40 days after treatment. Thenafter, a significant decrease in the production of kynurenic acid was observed 70 days after treatment (2.76 μmoles/g liver) compared with 4.83 μmoles/g liver for controls. Moreover, a significant decrease in the production of anthranilic acid was observed from the tenth day of treatment up to the end of this study (0.54, 0.49 and 0.41 μmoles/g liver) compared to the corresponding control values (1.63, 1.42 and 2.0 μmoles/g liver).

In the infected group of mice (Group III, Table 1) a slight decrease in the production of kynurenic acid was firstly observed which then significantly decreased from controls 120 days after infection. On the other hand, the production of anthranilic acid was significantly decreased directly after 60 days of infection and gradually increased by duration until it reached a normal level (Group III, Table 1).

In the infected group of mice treated with oxamniquine (Group IV, Table 1) the statistically significant decrease in the production of anthranilic acid encountered 10 days after treatment was observed again 70 days after treatment. The decrease in kynurenic acid production was observed lately 70 days after treatment.

DISCUSSION

Results of this study indicate that the two systems responsible for kynurenine metabolism, i.e. kynurenine hydrolase and kynurenine aminotransferase, were inhibited by oxamniquine. The inhibition encountered in the hydrolase enzyme was observed from the tenth day of treatment. This inhibition in the hydrolase enzyme is permanent only in the treated groups; therefore we can conclude that the inhibitory effect of the drug on the hydrolase enzyme is permanent and more prolonged than the effect of infection itself.

The effects of oxamniquine and *S. mansoni* infection in inhibiting the kynurenine aminotransferase are identical. This inhibition encountered in the aminotransferase enzyme was observed lately, 120 days after infection (70 days after treatment) in all groups studied. From these results it is obvious that kynurenine hydrolase is more sensitive to oxamniquine therapy than kynurenine aminotransferase because the inhibition of the former enzyme precedes that of the latter.

In a previous work [3] the inhibition in these B_6-dependent enzymes observed in *S.mansoni* infection was explained by the observation that *S.mansoni* worms created a deficiency in pyridoxal phosphate of the infected mouse liver. This effect was discussed in view of the possibility of two combined mechanisms: (a) an antimetabolite secreted by the infected worms or present in its eggs partially inhibited the phosphorylation of pyridoxal phosphate; (b) the concentration effect of the worms on the pyridoxal phosphate which results in a reduced level of the cofactor.

It was previously reported on the effect of some antimonial antibilharzial drugs [3, 4, 8, 13] that the antimonial content of these drugs is the factor responsible for the inhibition encountered in the kynurenine hydrolase and kynurenine aminotransferase enzyme systems. The possibility of the formation of an inactive chelate between antimony and pyridoxal phosphate, which is the needed cofactor in both enzyme reactions, was offered as a mechanism for the observed inhibition [3, 8]. The inhibition induced by oxamniquine may be explained by the presence of the hydroxymethyl group in oxamniquine (Fig. 1) and its metabolite, since this group in pyridoxal provides a site of attachment for the ester phosphate in the process of pyridoxal phosphate formation [14]. Thus, oxamniquine with its hydroxymethyl group may interfere and supress the process of pyridoxal phosphate formation.

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Effects of dipyridamole on human blood lymphocytes*

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Dipyridamole [2,6-bis(diethanolamino)-4,8-dipiperidino (5,4-d)pyrimidine] inhibits the transport of purine and pyrimidine nucleosides through the membranes of normal and malignant mammalian cells [1]. This effect of dipyridamole was demonstrated in human erythrocytes [2], platelets [3] and lymphocytes [4], pig vascular endothelium [5], chicken fibroblasts [6], heart [7], murine leukemic lymphoblasts [8] and rat hepatoma cells [9]. Concentrations of dipyridamole inhibiting the influx of nucleosides into cells were lower than those altering the output [8].

We have examined the effects of dipyridamole on the uptake of [^{3}H]thymidine into human blood lymphocytes, on the stimulation of lymphocytes by PHA and on the formation of rosettes with sheep red blood cells (SRBC).

Lymphocyte suspensions. Human blood lymphocytes from healthy volunteers were isolated on a Ficoll–Hypaque gradient. Macrophages were removed by previous 30 min stirring of heparinized blood with carbonyl iron (2 mg/ml, Calbiochem AG, Lucerne, Switzerland). Lymphocytes were washed three times and suspended in Parker's medium. Platelet contamination was less than one platelet per nucleated cell.

[^{3}H]*Thymidine uptake by lymphocytes.* Lymphocyte suspensions (200 μl, containing 2.5 × 10^6 cells) were incubated with 200 μl of Parker's medium alone or containing dipyridamole (Pharma Research Canada Ltd., Quebec) and 0.2 μCi (10 μl) of [^{3}H]thymidine (2 Ci/mole, UVVV Prague, Czechoslovakia) at 37°. At the time intervals indicated, lymphocytes were spun down by centrifugation at about 700 *g* for 5 min and washed three times with Parker's medium. The cell pellets were solubilized in 0.5 ml of Nuclear Chicago Solubilizer (Amersham/Searle, U.S.A.) during overnight incubation at 37°. Radioactivity of cell lysates was measured by liquid scintillation spectrometry [10].

Incorporation of [^{3}H]*thymidine and* [^{14}C]*leucine into lymphocyte cultures.* Lymphocyte suspensions (200 μl, containing 2.5 × 16^6 cells) in Parker's medium supplemented with horse serum (15% v/v), glutamine (3% w/v) and canamycin (3 μg/ml) were cultivated using a microculture system in a humidified atmosphere of 95% air and 5% CO_2 in an ASSAB incubator at 37° for 72 hr. Dipyridamole in Parker's medium (20 μl) and 1 μl of PHA (Difco, Detroit, MI) were added before cultivation. [^{3}H]Thymidine (0.2 μCi/10 μl) or [^{14}C]leucine (0.1 μCi–62 mCi/mmole, Amersham/Searle, U.S.A.) in 20 μl was added 18 hr or 4 hr before cell harvesting, respectively. Incorporation of [^{3}H]- or [^{14}C]-radioactivity into acid-insoluble material was determined by liquid scintillation spectrometry [10].

Rosette tests. Rosettes formed during incubation of lymphocytes with sheep red blood cells (SRBC) at 4° for 1 hr were assayed according to Jondal *et al.* [11] and called E-late. The technique of Wybran and Fudenberg [12] was applied for examination of rosettes E-early formed immediately after mixing lymphocytes with SRBC at room temperature. Rosettes EA formed by Fc-receptor bearing cells were investigated as described by Benwich *et al.* [13] using SRBC sensitized with the maximal subagglutinating dose of anti-SRBC rabbit antibody (Biomed, Kraków, Poland). Dipyridamole dissolved in Hank's solution, pH 6.8, was added in a volume not exceeding 20 per cent of the total volume of the samples tested. Percentages of rosettes were calculated after counting one thousand lymphocytes.

Binding of [^{14}C]*dipyridamole to lymphocytes.* Lymphocyte suspensions (200 μl, containing 5–10 × 10^6 cells) in Hank's solution, pH 6.8, were incubated with 200 μl of [^{14}C]dipyridamole (7.3 μCi/mg, Pharma Research Canada Ltd., Quebec, Canada) dissolved in the same solution.

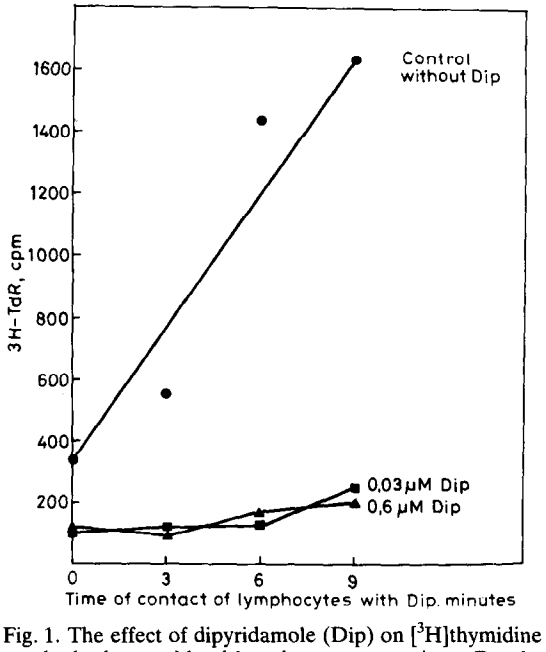

Fig. 1. The effect of dipyridamole (Dip) on [^{3}H]thymidine uptake by human blood lymphocyte suspensions. Results are mean values from determinations in four to five samples. For details see Materials and Methods.

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