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Letter to the editors

Homocysteine and the methotrexate toxicity in trisomy 21*

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Sir,

The "homocysteine hypothesis" in trisomy 21, put forward by Ueland et al. [17], prompted us to submit a discussion of an experiment performed in our laboratory in 1986 [9].

A possible abnormality of the homocysteine pathway in trisomy 21 has long been suspected [6]; the brachymorphic habitus of Down's syndrome is quite the countertype of the slender, Marfan-like phenotype of homocystinuria [7]. This clinical reasoning was corroborated in 1984 by the localization of the gene of cystathionine beta-synthase on chromosome 21 [16] and the expected gene/dosage effect for the enzymatic activity was demonstrated the following year [2]. Around this time, Peeters and Poon [12] and Peeters et al. [10] found methotrexate to be twice as toxic to children with trisomy 21 than to other children when given as a treatment for leukemia. This remarkable phenomenon has been repeatedly confirmed [1, 4, 5, 14].

In 1986 we developed a systematic experiment comparing methotrexate in vitro toxicity to lymphocyte cultures derived from healthy Down's syndrome-affected children and from their normal siblings [9]. The mitotic index was estimated in >3,000 lymphocytes/slide. Cells were harvested in the classic way after 72-h cultures to which various doses of methotrexate had been added (0, 0.6, 1.2, 2.4, 4.8×10^{-8} M). L-Homocysteine (100 or 200 mg/l) and L-methionine (200 mg/l) were also added to some of these cultures. The results are summarized in Table 1.

The percentage of diminution of the mitotic index was roughly proportionnal to the square of the dose of methotrexate, and the sensitivity of lymphocytes with trisomy 21 was twice that of normal cells. Since the original data obtained on 150 different lymphocyte cultures from 6 children with trisomy 21 and 6 of their healthy siblings, this hypersensitivity has been constantly confirmed in our laboratory. In all, >50 patients have shown this methotrexate sensitivity.

Table 1. Mitotic index of lymphocyte cultures

MTX ($\times 10^{-8}$ M)	$n=6$	+HCYS ($n=6$)	+MET ($n=3$)	$n=6$	+HCYS ($n=6$)	+MET ($n=3$)
0	62±24	55±18	63±14	65±18	66±25	70±8
0.6	49±20	51±28	56±20	64±17	66±16	65±4
1.2	32±22	41±28	31±16	55±21	61±17	58±28
2.4	5±6	7±6	19±16	35±19	33±22	21±12
4.8	4±4	3±5	6±5	4±4	7±5	8±6

Mean (±SD) values are expressed as the mean number of mitoses recorded per 1,000 cells after scoring of at least 3,000 cells. Various concentrations of methotrexate (MTX) were added (0, 0.6, 1.2, 2.4, 4.8×10^{-8} M). Six cultures were done for each dose of MTX. For each MTX dose, homocysteine (+HCYS) was also added to six additional cultures and methionine (+MET), to yet three others (data from Leujeune et al. [9]). The three left columns show trisomic data, the three on the right show data for normal controls

In accordance with previous discussions, the possible role of homocysteine and of methionine was investigated. At millimolar concentrations, neither homocysteine nor methionine, had any appreciable effect, as shown in Table 1. Although the lymphocytes with trisomy 21 were twice as sensitive as those from healthy siblings, this highly significant phenomenon remained unchanged, regardless of the addition of homocysteine or methionine to the cultures. This was seen at each of the methotrexate doses.

As these findings were established in 25 cultures of trisomics and 25 of normals for homocysteine and 15 cultures of trisomics and 15 of normals for methionine, it seems to us that this experiment gave an answer in advance, and negatively, to the hypothesis of Ueland et al. [17].

This does not mean that homocysteine leakage toward cysteine (due to the 1.5-fold higher activity of cystathionine beta-synthase) does not play a major role in Down's syndrome. The clinical findings cited above require a careful analysis of the situation.

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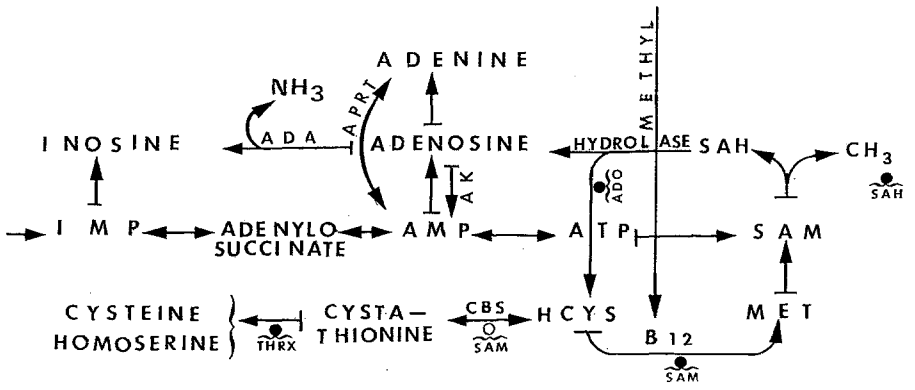

Fig. 1. Homocysteine and adenosine pathways

As shown in Fig. 1, at each methylation step S-adenosyl-methionine (SAM) becomes S-adenosyl-homocysteine (SAH), which is cleaved (hydrolase) into adenosine (ADO) and homocysteine (HCYS). By acceleration of cystathionine beta-synthase (CBS), HCYS is depleted [3] and cysteine is increased [18]. Depletion of HCYS would accelerate hydrolase and increase adenosine, but moderately, because adenosine itself blocks this reaction. As discussed by Lejeune [8], the pharmacological effects of adenosine could mimic some of the biochemical traits found in Down's syndrome.

As adenosine is also an inhibitor of the synthesis of phosphoribosyl pyrophosphate and of uridine monophosphate, the two limiting steps of purine and pyrimidine synthesis could be partly inhibited, hence the hypersensitivity to methotrexate. A direct action on thymidine synthesis seems less likely: FUdR toxicity and thymidine rescue efficiency are comparable in normal lymphocytes and in those with trisomy 21 [11, 15]. Purine (and pyrimidine) regulation seems to be very much worth investigating in trisomy 21 [10], and research along these pathways is in progress.

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