

Influence of stored aqueous ceftriaxone solutions on colony formation by neutrophil and macrophage precursor cells

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Aqueous solutions of ceftriaxone in concentrations similar to those achieved in man and stored at temperatures of 4°C, 22°C and 37°C for up to four weeks were tested in methylcellulose cultures of normal bone marrow from ten donors.

No significant differences were detected between the colony formation by neutrophil and macrophage precursors in control cultures and in cultures containing stored ceftriaxone.

It is concluded that aqueous ceftriaxone solutions stored for up to four weeks at temperatures up to 37°C are not altered to such an extent that they adversely affect colony formation by bone marrow progenitor cells from healthy donors.

Introduction

Penicillin and cephalosporin derivatives have been reported to induce neutropenia and it has been claimed that degradation products of these antibiotics are responsible for this side effect (Müller *et al.*, 1983).

We therefore studied the influence of stored ceftriaxone, a semi-synthetic cephalosporin, on colony formation of myeloid precursor cells *in vitro*, using the methylcellulose assay.

Materials and methods

Ceftriaxone solutions

A sterile ceftriaxone (Rocephin) solution was prepared at a concentration of 1 mg/ml in redistilled water and stored for up to four weeks at 4°C, at room temperature (approx. 22°C) and at 37°C. Aliquots of this solution were appropriately diluted and tested on bone marrow myeloid precursor cells as described below.

Bone marrow cultures

Cryopreserved nucleated bone marrow cells 10⁵/ml, with approximately 90% viability as determined by trypan-blue exclusion, obtained from ten bone marrow transplantation donors, were incubated in 5% CO₂ at 37°C in a humid atmosphere in Iscove's modified Dulbecco's medium (Gibco, E 096550). This medium

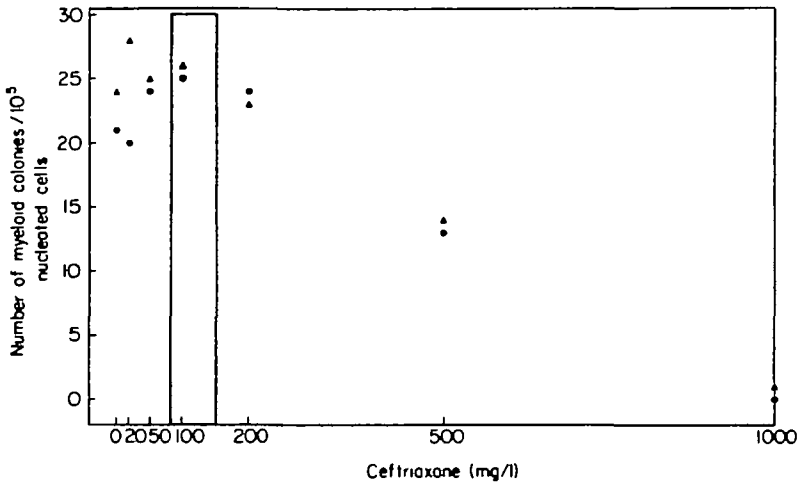


Figure 1. Effect of freshly prepared ceftriaxone on colony formation by neutrophil and macrophage precursors. ▲, Result of experiment 1 (two bone marrow samples); ●, result of experiment 2 (two bone marrow samples). Stippled = therapeutic plasma ceftriaxone concentration (peak plasma concentration obtained 30 min after iv infusion of 0.5 and 1 g of ceftriaxone).

contained 0.8% methylcellulose, 16% fetal calf serum (Gibco), 1% de-ionized and delipidated bovine serum albumin, 360 mg/l of human transferrin (Otre) and 16% v/v human PHA-induced leucocyte conditioned medium (CM). After 14 days, colonies formed by myeloid precursor cells were counted and differentiated with the aid of an inverted microscope.

Evaluation and statistics

Results are expressed as arithmetical mean and standard error of the mean (S.E.M.). For statistical evaluation the non-parametric two-way analysis of variance according to Friedmann (Siegel, 1956) was used.

Results

As shown in Figure 1, freshly prepared ceftriaxone did not inhibit proliferation of myeloid precursor cells in concentrations up to 200 mg/l, while colony growth was reduced to about 50% by 500 mg/l and totally abolished by 1000 mg/l of ceftriaxone. Furthermore, no colony growth was observed in cultures containing no ceftriaxone and no CM.

Based on these results, concentrations of 100 mg/l of ceftriaxone were added to the cultures in the subsequent storage experiments. Neither storage at room temperature (22°C), nor at 4°C or at 37°C for up to 96 h revealed any adverse effect on myeloid colony growth (Table I), even though a slight yellowish colour was observed in aqueous ceftriaxone solutions kept for 24 h at 37°C. During 24 h, degradation of ceftriaxone in solution amounted to 1–3% at 22°C and up to 9% at 37°C. Correspondingly, 1.1–2.1% and 4.3%, respectively, of decomposed active substance was formed at these storage temperatures.

Table I. Comparison between colony formation by neutrophil and macrophage precursors in control cultures and in cultures containing aqueous ceftriaxone solutions stored at various temperatures for up to 96 h

Assay		Number of donors	Number of myeloid colonies (cfu-c)/10 ⁵ nucleated cells	S.E.M.	Computed α (preassigned $\alpha = 0.050$)
No ceftriaxone		10	33.2	3.02	
+ ceftriaxone	(0.5 h/22°C)	10	34.4	2.13	0.972 = n.s.
+ ceftriaxone	(48 h/22°C)	10	34.1	2.12	
+ ceftriaxone	(96 h/22°C)	9	34.6	2.72	
No ceftriaxone		10	35.3	2.83	
+ ceftriaxone	(0.5 h/ 4°C)	10	33.7	2.42	0.463 = n.s.
+ ceftriaxone	(48 h/ 4°C)	10	34.7	2.69	
+ ceftriaxone	(96 h / 4°C)	9	34.8	2.35	
No ceftriaxone		10	34.3	2.56	
+ ceftriaxone	(0.5 h/37°C)	10	35.8	2.34	0.339 = n.s.
+ ceftriaxone	(48 h /37°C)	10	33.8	2.33	
+ ceftriaxone	(96 h/37°C)	9	34.8	2.21	

n.s., No significant differences between colony counts resulting from different storage conditions and those of the controls.

In order to detect possible effects of prolonged storage, aqueous ceftriaxone solutions kept for two, three and four weeks at the three temperature levels were additionally tested on bone marrow cultures from a small number of donors. Again, myeloid colony growth remained unaffected by the stored ceftriaxone and the results obtained were in good agreement with those of the control cultures. After aqueous ceftriaxone solutions had been stored for four weeks and then tested on bone marrow cultures from two donors, the following numbers of myeloid colonies/10⁵ nucleated cells were obtained (mean \pm S.E.M.): controls = 39.0 \pm 0, stored ceftriaxone (22°C) = 38.0 \pm 2.0; controls = 39.0 \pm 6.0, stored ceftriaxone (4°C) = 38.5 \pm 4.5; controls = 40.0 \pm 1.0, stored ceftriaxone (37°C) = 39.5 \pm 0.5).

Discussion

Neutropenia has occasionally been reported following treatment with high doses of ceftriaxone, but as yet no proof of a causal relationship between the adverse reaction and the medication has been produced. The incidence of this side effect during treatment with ceftriaxone is extremely low and amounts to 0.35–0.54% of the patients according to literature reviews covering some 1347 publications and presentations and up to 8565 patients (Havas, Fernex & Kissling, 1983; Fernex, M., Havas, L. & Kissling, M., unpublished results). Comparative trials with 4100 patients given ceftriaxone and 4238 patients given standard antibiotic therapy revealed identical incidences of neutropenia in both groups.

However, in view of the potential therapeutic advantage of parenteral out-patient therapy in rural areas using pre-prepared ceftriaxone solutions, proof was desired that stored ceftriaxone solutions do not alter to such an extent that they adversely affect

colony formation by myeloid bone marrow precursor cells and thus induce neutropenia.

The use of Iscove's seme-solid culture method was the method of choice as it has been successfully applied by other authors to investigate similar problems (Negishi & Young, 1985). Even though our criteria in the study design were very strict, the added ceftriaxone solutions did not interfere with colony growth by myeloid bone marrow precursor cells from healthy donors.

This result is comparable to the findings reported by Osterwalder *et al.* (1986), who investigated whether or not therapy with cephalosporin alone or combined with other β -lactam antibiotics (even in doses in excess of 100 g in ten days or more) inhibited myelopoiesis *in vivo* in patients with allogeneic bone marrow transplants. These authors stated that no inhibitory influence of cephalosporin or combinations thereof with other β -lactam antibiotics on myelopoiesis was detectable in patients with severe aplastic anaemia and who had undergone bone marrow transplantation.

References

- Havas, L., Fernex, M. & Kissling, M. (1983). Literature survey on the clinical efficacy and tolerance of ceftriaxone: an analysis of 3961 cases. In *Proceedings on Progress in Therapy of Bacterial Infections. A New Cephalosporin: Ceftriaxone*, Asia Pacific Congress Series No. 19, pp. 130-45. Excerpta Medica, Amsterdam.
- Müller, M., Neftel, K., Wälti, M., Hauser, S., Erni, J. & Arrenbrecht, S. (1983). Semisynthetische Penicilline und Cephalosporine hemmen die In-vitro-Myelopoiese dosisabhängig. *Schweizerische Medizinische Wochenschrift* 113, 1471-3.
- Negishi, M. & Young, C. (1985). Effects of ofloxacin on the haemopoietic stem cells and comparison with other antimicrobial agents. In *Recent Advances in Chemotherapy, Antimicrobial Section* (Ishigami, J., Ed.), pp. 861-2. University of Tokyo Press.
- Osterwalder, B., Haberthür, C., Gratwohl, A. & Speck, B. (1986). Können Betalactam-Antibiotika (BLA) die Myelopoiese *in vivo* hemmen? Eine Studie bei 115 Patienten mit allogener Knochenmarktransplantation (KMT). *Schweizerische Medizinische Wochenschrift* 116, Suppl. 20, 26.
- Siegel, S. (1956). The Friedmann two-way analysis of variance. In *Nonparametric Statistics for the Behavioral Sciences*, Siegel, S., Ed.), pp. 166-73. McGraw-Hill, New York.

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