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Cell Kinetics with *in vivo* Bromodeoxyuridine and Flow Cytometry: Clinical Significance in Acute Non-lymphoblastic Leukaemia

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From 1986 to 1988, 54 consecutive previously untreated patients with acute non-lymphoblastic leukaemia (ANLL), median age 54 years, were treated for remission (CR) induction with vincristine and intravenous medium-dose cytarabine sequentially followed by daunomycin and infusion cytarabine. CR patients received intensive consolidation. Bone marrow blast kinetics was studied before therapy with *in vivo* bromodeoxyuridine and bivariate flow cytometry. CR rate was 70.2%, median CR was 13.2 months, responsive patient survival was 16.9 months and overall survival was 9.2 months. Besides lower median age, the 33 responsive patients also had shorter potential doubling time (T_{pot}) and greater cell production rate (PR) than the 14 unresponsive patients (mean values = 10.9 vs. 25.4 days, $P < 0.05$, and 14.7 vs. 8.9 cells/100 cells/day, $P < 0.02$, respectively), due to a higher mean labelling index (7.0 vs. 5.1%, $P < 0.05$) and/or to a shorter mean DNA synthesis time (13.6 vs. 18.6 hours, $P < 0.05$). Besides lower white blood cell count and bone marrow blast percentage, patients who experienced CR longer than 13.2 months had shorter T_{pot} ($P < 0.05$) and a greater PR ($P < 0.02$) than those who relapsed before this time. These data indicate that kinetic parameters have prognostic relevance in ANLL patients treated with sequential vincristine, cytarabine and daunomycin for inducing CR and with intensive consolidation after CR, a high proliferative activity being a favourable factor for both CR achievement and its duration.
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INTRODUCTION

IN THE LAST decade no major advance has been made in the treatment of adult acute non-lymphoblastic leukaemia (ANLL), mainly because no cytostatic having greater effectiveness than cytarabine and daunomycin on the leukaemic population has become available [1]. Studying the cell kinetics of bone marrow blasts and manipulating it could offer some practical information and advantages. For example, remission (CR) rate is possibly greater with high S-phase values [2], especially when chemotherapy is accomplished with S-phase specific drugs [2,3]. Besides, in *in vitro* experiments, increasing the aliquot of S-phase blasts with colony stimulating factors (CSFs) has been reported to increase the cytocidal capacity of subsequently administered cytostatics [4]. The intravenous push administration of vincristine and of medium dose cytarabine seems to synchronise blasts in S-phase [5, 6], and this, in turn, increases the cytocidal effectiveness of a sequentially administered anthracycline [5].

We report a prospective study on ANLL where vincristine, cytarabine and daunomycin were sequentially given to induce CR, according with the above cytokinetic suggestions [5]. An intensive consolidation program (as operationally defined as able to produce, in a CR patient, significant cytopenia, i.e. a white blood cell (WBC) count of less than 1.0×10^9/l and a platelet count of less than 50×10^9/l, about 2 weeks following its completion) [7] was administered to CR patients [8].

The pretreatment ANLL kinetics was detailed with *in vivo* bromodeoxyuridine (BUDR) and flow cytometry (FCM) [9]. This procedure allows experimental determination of the labeling index (BUDR-LI) and of the duration of S-phase (i.e. the DNA synthesis time, TS), from which other kinetic parameters are calculated [10] that are meaningful in giving the overall picture of ANLL growth, namely the potential doubling time (T_{pot}), i.e. the time at which the whole cell population doubles, and the cell production rate (PR).

PATIENTS AND METHODS

This prospective, non-randomised study started in January 1986, and closed in December 1988, with 54 consecutive, previously untreated ANLL patients entering it by this time. No patient with a diagnosis of ANLL was excluded due to advanced age, poor general condition or history of preleukaemia. Patients had FAB classification [11] and conventional cytogenetics [12] performed. The bone marrow blast kinetics was studied with *in vivo* BUDR before they received induction treatment. Authorisation for this procedure was given by the ethical committee of the Department of Internal Medicine of the University of Pavia and written informed consent was obtained from each patient.

Cytostatic therapy: induction treatment

Two courses of induction treatment were planned at a 14–21 day interval. Drug scheduling was derived from previous *in vivo* data [5, 6], suggesting that the intravenous push administration of vincristine and medium dose cytarabine increased the tritiated thymidine labelling index in a significant percentage of acute

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leukaemias, and this ameliorated the effectiveness of a sequentially administered anthracycline in reducing the peripheral blood blasts [5].

In each course, patients initially received vincristine (2 mg intravenously on day 1) and cytarabine (75 mg/m^2/12 h directly intravenously from days 1 to 4). Daunomycin (80 mg/m^2, intravenously) was then given on days 5 and 7 and cytarabine (200 mg/m^2 per day by continuous infusion) on days 6 and 7. Bone marrow aspirate was performed 10–14 days after each course. CR was defined as disappearance of bone marrow blasts with normal haematological values (granulocytes $> 1.5 \times 10^9$/l, platelets $> 100 \times 10^9$/l and haemoglobin > 11 g/dl) maintained for at least 1 month without transfusion.

Consolidation therapy

CR patients received intensive consolidation with four courses of therapy including some cytostatics not used for induction. In each consolidation course, they were given sequentially vincristine and cytarabine as in the induction course. In practice, vincristine and intravenous cytarabine were followed by etoposide (100 mg/m^2 per day by 2-h intravenous infusion) + 6-thioguanine (100 mg/m^2 per day, orally) for 5 days (1st and 3rd courses) or by amsacrine (60 mg/m^2 by 2-h intravenous infusion) on day 5 (2nd and 4th courses).

Post consolidation treatment

Following consolidation, CR patients were randomised to receive or not receive a maintenance program, in which different cytostatics were given every 4–6 weeks in order to circumvent drug resistance in residual leukaemia [13–15]. The following drugs and drug combinations were used: TRAP (courses 1, 6 and 11) [14], etoposide (150 mg/m^2 per day, intravenously, days 1–5, courses 2, 7 and 12), POMP (course 3) [14], cytarabine + 6-thioguanine (cytarabine, 100 mg/m^2 every 12 h intravenously + 6-thioguanine, 100 mg/m^2 per day orally, days 1–5, courses 4, 9 and 14), COAP (courses 5, 10 and 15) [14], lomustine (80 mg/m^2 orally, day 1, course 8) and cyclophosphamide (1 g/m^2 per day by 2-h intravenous infusion, days 1 and 2, course 13).

CR duration is calculated from the date of CR to the date of relapse. Survival is calculated from the start of therapy.

Supportive measures

Patients were maintained in conventional hospital isolation in single rooms, and antibacterial (co-trimoxazole or norfloxacin) and antifungal (myconazole) prophylaxis was started before therapy in non-febrile patients after culture samples were drawn. Empirical (with aztreonam associated with clindamycin) or antibiogram directed (in patients with positive cultures) antibiotic therapy was instituted in febrile patients ($t > 38$°C for at least 2 consecutive days). Granulocyte transfusions from unrelated or related donors were also administered daily if a 3-day course of antibiotic therapy was ineffective in granulocytopenic patients (granulocytes $< 0.5 \times 10^9$/l). Platelet transfusions were administered only if haemorrhage occurred. Haemoglobin was maintained over 10 g/dl.

Cell kinetics

In order to have a comprehensive picture of bone marrow blast kinetics, blast cell LI and DNA synthesis time (TS) were experimentally determined with *in vivo* BUDR and bivariate FCM, from which the T_{pot} of the population and its PR were calculated. This procedure requires that patients receive a BUDR infusion (to label S-phase cells), that a BM sample be obtained 4–6 hours following BUDR infusion, that the whole cell population be double stained for BUDR incorporation and DNA content, and that bivariate FCM be used to determine simultaneously the distributions of BUDR labeling versus DNA content. The whole method takes 8–9 hours from the start of BUDR infusion. It has been detailed elsewhere [9] and is summarised here.

BUDR (500 mg in 100 ml NaCl) was infused in 20 minutes and bone marrow was sampled 4–6 hours after completion of BUDR infusion.

A 0.1–0.5 ml bone marrow sample (containing 92–100% of blasts) was obtained by vigorous aspiration from the sternum and placed in a watch glass containing a few drops of sodium citrate, from which single bone marrow particles were picked up by a sharp thumb forcep [16]. They were gently washed in saline, placed into a 5 ml tube containing 1 ml of phosphate-buffered saline (PBS) and finally disrupted by pipetting, to obtain a single cell suspension [17]. Cells were layered (in a ratio of 1:1) on Ficoll–Hypaque, collected after centrifugation (6.0 *g* for 30 min), washed twice in PBS, filtered through a 35-μm pore nylon filter, resuspended in PBS and ultimately fixed in 70% ethanol at a concentration not exceeding 1×10^6 cells/ml.

For detection of BUDR incorporation, double stranded DNA must be denatured with 2 mol/l HCl in order to allow the anti-BUDR Mab (Becton Dickinson) to react with the BUDR in the DNA chain. The reaction is then visualised by means of a standard immunofluorescence technique using a goat anti-mouse IgG FITC conjugate (Sigma). Cells are later stained for DNA content by resuspending them in 4 ml PBS containing 10 μg/ml propidium iodide (which stechiometrically stains the nucleic acid).

Bivariate distribution of BUDR labeling (green) versus DNA content (red) was determined with a FACS Star Cell Sorter (Becton Dickinson), and data were analysed using a dedicated Hewlett Packard Computer. For each bone marrow sample 20 000–50 000 cells were measured.

The LI and TS determination from FCM histograms assumes that at the time of BUDR infusion the mean DNA content of BUDR-labeled S-phase cells is in the middle of the interval between the 2*n* (G1/0) and 4*n* (G2) peaks (as determined using normal BM cells as diploid reference standard) and that the rate of cell progression through the S-phase is constant. At the time of BM sampling, i.e. 4–6 hours later, the S-phase cells (which were labeled at the time of BUDR infusion) have moved toward G2 at a rate that is dependent on their TS, so that their peak DNA content distribution appears as shifted toward 4*n*.

From these cytograms, the LI value is the percentage of the BUDR-labeled S-phase cells over the whole population. The TS is calculated by determining the mean DNA content of the BUDR-labeled S-phase cells. In fact, their position (shifted toward G2) allows one to determine the rate at which these cells have progressed through the S-phase in the interval between BUDR infusion and tumour sampling. The TS is the time S-phase cells are expected to spend in reaching G2 at this progression rate. In both LI and TS calculation, allowance was made for the small aliquot of S-phase cells that have recycled to G0/1 following mitosis in the time elapsed between BUDR infusion and BM sampling [9].

The T_{pot} and PR were calculated assuming a steady state condition [10]. The T_{pot} (days) is given by the formula: {(TS/LI) $\times$ 100}/24, and PR (cells/100 cells/day) is the reciprocal: LI/TS $\times$ 24.

Table 1. Pretreatment characteristics with prognostic significance for patients with acute non-lymphoblastic leukaemia who experienced remissions (CR) and survivals of different durations

				CR (mos)			CR patients survival (mos)		
Parameter	CR	NR	*P*	>13.2	<13.2	*P*	>16.9	<16.9	*P*
Age (yrs)	46	58	<0.005	53	57	ns	54	57	ns
WBC (10^9/l)	24	32	ns	12	45	<0.01	16	36	<0.005
BMB (%)	86	85	ns	75	94	<0.05	84	85	ns
LI (%)	7.0	5.1	<0.05	8.5	6.1	<0.05	8.2	6.3	<0.05
TS (h)	13.6	18.6	<0.05	11.4	13.9	ns	11.9	12.9	ns
T_{pot} (days)	10.9	25.4	<0.05	8.1	13.1	<0.05	8.3	12.7	<0.05
PR*	14.7	8.9	<0.02	20.4	11.1	<0.02	20.1	11.3	<0.02

*Cells/100 cells per day.
NR = no response, WBC = white blood cells, BMB = bone marrow blasts, LI = labeling index, TS = DNA synthesis time, T_{pot} = potential doubling time, PR = production rate.
Median values are reported for age, WBC and BMB and mean values are reported for kinetic parameters. For kinetic parameters that were not normally distributed, other descriptive statistics are given in the text.

Statistical analysis

The evaluation of the relationship between pretreatment kinetic (LI, TS, T_{pot} and PR) and clinical parameters (age, splenomegaly and hepatomegaly, as cm below the costal margin; fever, graded from 0 = absent to 5 = exceeding 40°C; haemoglobin level, g/dl; platelet and peripheral blood blast number, × 10^9/l; and BM blast, %) parameters and the course of ANLL (including response to chemotherapy and duration of first CR and of survival) was done in two ways: (1) by searching for a cutoff value for the above parameters which offered a statistically significant discrimination in CR rate and in duration of CR and of survival. Survival and remission duration curves were constructed according to the method of Berkson and Gage [18] and analysed by the Lee–Desu test [19]; and (2) by separating patient groups (according to response and durations of CR and of survival) which differed statistically (by the non-parametric Wilcoxon test) for the distribution of the above clinical and kinetic parameters.

In order to determine the features at diagnosis which were independently correlated with duration of response and of survival, multiple regression analysis of the above kinetic and clinical parameters was used according to the stepwise backward method of the Statistical Package for the Social Sciences (SPSS) program [20], implemented on a Honeywell DPS8 computer.

RESULTS

The results obtained are summarised in Table 1 and in Figs 1 and 2.

Treatment

Of 57 patients with ANLL, 54 (median age = 54 years, range: 20–78; M/F = 28/26) entered induction chemotherapy (3 patients died before therapy was started). 4 patients had had myelodysplasia before ANLL. Distribution of FAB subtypes was as follows: M1 (8 patients), M2 (10), M3 (7), M4 (21), M5 (5), M6 (2) and M7 (1). Chromosomal abnormalities were found in 10 patients (translocation 8/21, 2 patients; trisomy 8, 3 patients; monosomy 7, 2 patients; trisomy 11, 1 patient; inv 16 (p13 q22), 1 patient; and 1q+, 1 patient).

Excluding early deaths (7/54 = 12.9%), 47 patients completed induction. Median age of these patients was 56 yrs and the male/female ratio was 25/22. 33/47 (70.2%) patients achieved CR and 14 failed to respond due to resistant leukaemia. 76% of responsive patients achieved CR following the first course of therapy.

Median CR duration was 13.2 months (Fig. 1). 12 of the 33 CR patients relapsed before randomisation for maintenance. There was no difference in CR duration for the remaining patients dependent on whether or not they received maintenance. Median survival was 9.2 months for all and 16.9 months for responsive patients.

Fever above 38°C following the first induction course occurred in 34/47 courses, the nadir of WBC (0.35 × 10^9/l) occurred on day 10 following the end of treatment (WBC were <0.5 × 10^9/l for a median time of 12 days) and the nadir of platelets (19 × 10^9/l) occurred on day 14 following the end of treatment (platelets were <50 × 10^9/l for a median time of 15 days).

Toxicity during consolidation was quite severe: 68% of CR patients had granulocyte counts less than 1.0 × 10^9/l and/or platelet counts less than 50 × 10^9/l following one course (the cytopenia phase lasted a median time of 5 days); 48% had these low values following two courses and 21% following three to

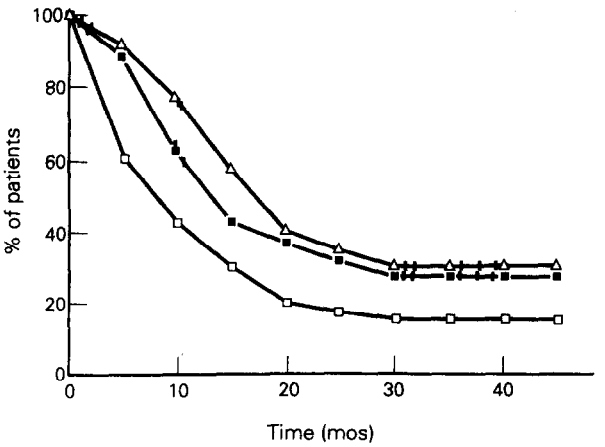

Fig. 1. Survival and remission (CR) curves for 54 patients with acute non-lymphoblastic leukemia. –□– Overall survival (*n* = 54), –■– CR duration (*n* = 33), –△– CR patients survival (*n* = 33).

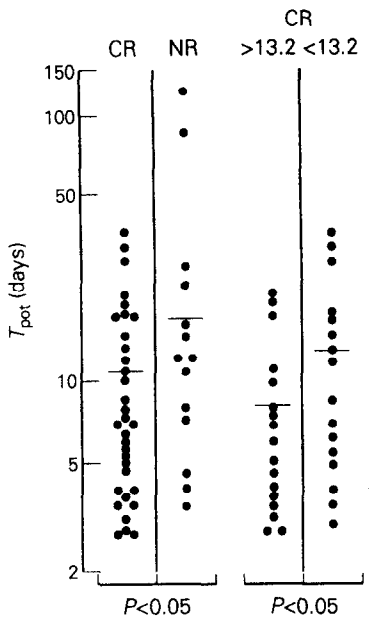

Fig. 2. Distribution of potential doubling times (T_{pot}) of bone marrow blasts in patients with acute non-lymphoblastic leukaemia who achieved remission (CR) or failed to respond to chemotherapy (NR) and in CR patients who experienced a CR longer (CR > 13.2) or shorter (CR < 13.2) than the median CR duration (13.2 months).

four courses. However, no patient died due to complications related to consolidation-induced cytopenia.

Kinetic characteristics

The LI and TS were successfully determined in the 47 patients who completed induction treatment, and there was no immediate toxicity from BUDR infusion. Mean LI was 6.2 (S.D. 2.9; median value = 5.3; range: 0.4–13.8)%, mean TS was 15.8 (S.D. 6.1; median value = 10.4; range: 6.5 = 37.5) hours, mean T_{pot} was 17.7 (S.D. 18.9; median value = 10.4; range: 2.7–122.9) days and mean PR was 12.1 (S.D. 7.9; median value = 9.9; range: 0.8–33.9) cells/100 cells/day.

Kinetic data were also obtained from 2 of 3 patients who died while untreated and from the 6 of 7 patients who died during induction. For these 8 patients, mean LI was 5.7 (range: 0.6–10.2)%, mean TS was 12.9 (range: 7.9–43.1) hours, mean T_{pot} was 14.9 (range: 4.0–66.2) days and mean PR was 10.3 (range: 3.3–27.9) cells/100 cells/day. These value were no statistically different from those obtained in patients who completed treatment, they were not used in the subsequent statistical analysis.

On investigating the correlation between the pretreatment kinetic and clinical parameters and the course of ANLL, haemoglobin less or greater than 7 g/dl was shown to be associated with overall median survival of 7.5 and 16.6 mos, respectively ($P < 0.01$). No cut-off value for any other parameter was useful in discriminating response, CR duration or CR patient survival.

Table 1 summarises the clinical and kinetic parameters that were statistically different in patient groups categorised according to CR and different durations of CR and of survival.

Of the clinical parameters, age was lower in the 33 responsive patients than in the 14 unresponsive ones and WBC count and bone marrow blast percentage were lower in the CR patients whose remission lasted longer than 13.2 months than in those who relapsed before this time.

Proliferative activity was higher in both CR patients and in those who experienced the longest durations of CR and survival. In fact, mean [S.D.] T_{pot} was shorter (10.9 [8.4] vs. 25.4 [24.1] days; median values = 7.5 vs. 12.0 days; ranges = 2.7–36.7 vs. 3.6–122.9 days, $P < 0.05$) (Fig. 2) and mean [S.D.] PR greater (14.7 [8.1] vs. 8.9 [6.7] cells/100 cells per day; median values = 13.3 vs. 6.8 cells/100 cells per day, ranges = 2.7–33.9 vs. 0.8–27.5 cells/100 cells per day, $P < 0.02$) in responsive than in unresponsive patients, due to a higher mean LI (7.0 vs. 5.1%, $P < 0.05$) and/or to a shorter mean DNA synthesis time (13.6 vs. 18.6 h, $P < 0.05$). CR patients with remissions longer than 13.2 months had a significantly shorter mean T_{pot} (8.1 vs. 13.1 days; median values = 6.0 vs. 10.2 days; $P < 0.05$) (Fig. 2) and a greater mean PR (20.4 vs. 11.3 cells/100 cells per day; $P < 0.02$) than those who relapsed before this time. Patients who survived longer than 16.9 months also had shorter T_{pot} and greater PR than those who died earlier (Table 1).

From multiple regression analysis, only the WBC count at diagnosis maintained an independent, statistically significant (inverse) correlation with duration of CR and of CR patient survival ($P < 0.05$, $r^2 = 14\%$).

DISCUSSION

In this investigation adult patients with ANLL underwent a prospective treatment and had detailed kinetic study with *in vivo* BUDR and FCM before chemotherapy was started. That kinetic parameters have clinical relevance is suggested by the fact that both the CR rate was greater and the CR duration and CR patient survival were longer in ANLL with shorter T_{pot} and greater PR, i.e. with high proliferative activity.

The results of induction treatment were sufficiently good, since it was both well tolerated and effective in inducing CR. The 12.9% early deaths is acceptable during induction for adult ANLL [1]. A 70.2% CR rate (calculated after exclusion of early deaths [21] compares well with data from recent investigations [1], especially if we consider that the median age was much higher in this study (54 yrs) than in most reported series and that the 74% of CR were achieved following the first course [1]. Post induction therapy was both intensive [7] and included drugs (amsacrine, etoposide and 6-thioguanine) not previously used for induction. Median CR duration was 13.2 months and the median survival of responsive patients was 16.9 months. These figures are better than those from a preceding protocol [8], where sequential vincristine, cytarabine and daunomycin were followed by non-intensive consolidation for CR patients. This is in keeping with at least some data [22] from a controversial literature [1] that intensive consolidation prolongs CR.

As expected [1], there was a poor relationship between conventional clinical and haematological parameters and patient prognosis. Only the pretreatment WBC count was consistently correlated (also in multiple regression analysis) with CR duration and CR patient survival.

Some prognostic information was added by the recently developed study of the *in vivo* BUDR incorporation, using bivariate FCM. This procedure allows experimental determination of LI and TS, from which the time for whole cell population duplication, namely the T_{pot}, and its rate of cell production, namely the PR, derived. The method has been thoroughly discussed elsewhere [9]. Briefly, the conclusions were that both BUDR-LI and BUDR-TS are reliable, since they give values consistent with those obtained with other methods. In fact, the FCM-BUDR-LI and the traditional *in vitro* [^{3}H]-TdR-LI or BUDR-LI are very close, both in experimental models [23, 24] and in humans [9, 25]. The BUDR-TS are

strictly related to the values we obtained on duplicate samples from quantitative [^{14}C]-TdR autoradiography [26] and to values obtained with other autoradiographic techniques [10, 27].

A higher proliferative activity was found in patients with a more favourable course of the disease. In fact, mean T_{pot} was shorter and mean PR was greater in responsive than in non-responsive patients and in those who experienced CR for over 13.2 months than in those who relapsed before this time, due to a higher mean LI and/or to a shorter mean TS.

The positive relationship between high proliferative activity and achieving CR may depend in part on the cytostatic protocol we applied. Highly proliferating ANLL can reach CR more often than the slowly proliferating ones because for the first 4 days two phase-specific drugs (vincristine and cytarabine) are given both of which preferentially kill proliferating cells [3] and probably increase the aliquot of S-phase residual blasts before daunomycin and continuous infusion cytarabine are administered on days 5–7 [5].

However, we believe that these data on the prognostic implications of cell kinetics in ANLL have general clinical relevance. Past investigations on this topic mainly included low numbers of patients, used greatly different induction and postinduction treatments (only 3 ANLL series were both sufficiently large and uniformly treated) [2, 3, 28] and considered the S phase (with 3H-TdR cytoautoradiography or DNA FCM) as the only parameter describing proliferation. Despite these biases, most of them [2, 3, 29–33] pointed out that a high S-phase favours CR, some [34–39] failed to find a CR advantage that depended on the S-phase and no one suggested a CR advantage for patients with low S-phase. In the present series, the BUDR incorporation and DNA distribution analysis demonstrate that not only a high S-phase but also a short TS may account for the greater overall proliferative activity in CR patients. A CR advantage for patients with shorter TS was reported with the 3H- and 14C-TdR double labeling technique [40], or with *in vivo* BUDR coupled with immunocytochemistry [41]. In some past studies [34–39], a shorter TS was possibly responsible for a short T_{pot} and a high PR in CR patients, independently on S-phase.

Literature data on the correlation between cell kinetics and CR duration and CR patient survival are scanty [2]. As a guideline, CR duration depends on the bulk of residual disease after induction, and factors influencing it could still be induction treatment modalities and cell kinetics at diagnosis. Preferential killing of proliferating cells by cytostatics explains why highly proliferating ANLL had lower residual disease and longer CR than poorly proliferating ANLL. The longer survival of patients with high proliferative activity is easily explained by their greater probability of achieving CR and of its longer duration.

Of course, the prognostic significance of proliferative activity in ANLL must be confirmed, especially by using treatment programs other than ours and by performing a multivariate analysis of disease features on large series. The aid given by the *in vivo* BUDR method is that it allows one to include kinetic parameters in this analysis.

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Effect of Angiotensin II on Intermittent Tumour Blood Flow and Acute Hypoxia in the Murine SCCVII Carcinoma

Martin J. Trotter, David J. Chaplin and Peggy L. Olive

Effects of the vasoconstrictor angiotensin II on tumour microvascular perfusion and oxygenation were examined in the murine SCCVII carcinoma grown subcutaneously in C3H/He mice. Angiotensin II infusion (2 μg/kg/min) caused an increase in mouse arterial blood pressure from 85 (2) mm Hg (mean, S.D.) to 112 (7) mm Hg. During drug infusion, tumour red blood cell (RBC) flow (measured by laser doppler flowmetry) increased 206 (50%) ($P < 0.001$) in unanaesthetised animals and 305 (90%) ($P < 0.001$) in mice immobilised with ketamine and diazepam. As assessed using a fluorescent double-staining technique, angiotensin II reduced staining mismatch (indicative of intermittent blood flow) in SCCVII microvasculature from 8.1 (2.5%) of total vessels to 2.0 (1.3%) ($P < 0.001$). However, a large proportion of this reduction could be attributed to volume loading. Angiotensin II reduced but did not completely eliminate the radiobiological acute hypoxia which results from intermittent tumour vessel non-perfusion. We propose that angiotensin II improves tumour microcirculatory flow distribution via its systemic actions, by elevating perfusion pressure, thereby preventing collapse and/or temporary flow stasis in tumour microvessels.
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INTRODUCTION

SELECTIVE MANIPULATION of tumour blood flow using vasoactive drugs has been extensively investigated as a means to improve conventional methods of cancer diagnosis and treatment [1]. Modulation of tumour blood flow is undertaken with the goal of reducing or eliminating perfusion heterogeneity, a factor considered detrimental to successful radiation therapy and delivery of chemotherapeutic agents. Increases in tumour perfusion, especially if they produce a homogeneous flow distribution, could result in improved tumour oxygenation and drug delivery.

Tumour blood flow is very sensitive to changes in systemic blood pressure [2, 3]. Flow autoregulation is apparently absent; at perfusion pressures > 40 mm Hg, tumour blood flow is linearly related to mean arterial blood pressure [4, 5]. This passive pressure–flow relationship, together with evidence that vascular smooth muscle and adrenergic innervation are often