Banded Karyotypes from Bone Marrow: A Clinical Useful Approach

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Summary. We have developed a new protocol for the preparation of banded chromosomes from human bone marrow. This protocol incorporates new procedures with improvements in conventional ones to rapidly produce high quality banded karyotypes from bone marrow aspirates. Tissue culture is completely eliminated and replaced with a truly direct method of chromosome preparation in which a small amount of marrow is treated with a solution containing trypsin, hypotonic salts and colcemid (THC). The THC protocol, when compared with standard short term culture methods for marrow chromosome preparation, produces more extended and more readily banded chromosomes. Rapid banding is further facilitated by replacement of standard G-banding techniques with Wright's staining. These technical developments allow karyotypic analysis within 2-4 h after receipt of the specimen. The high quality and rapidity of the THC protocol have important implications for the clinical usefulness of cytogenetic analysis of bone marrow in studying congenital defects as well as leukemias and lymphomas.

Introduction

The specificity of chromosomal abnormalities associated with various forms of human leukemias and lymphomas is becoming recognized as a possible tool in the differential diagnosis and treatment of these diseases (Bloomfield et al., Golomb et al., 1978; Van den Berghe et al., 1978). However, clinical application of karyotypic analysis in these disorders is hampered by the generally poor quality of banded karyotypes from bone marrow preparations and by the lengthy methods of analysis that are most often necessary (for reviews, see Rowley, 1974; Tjio and Wheng-Peng, 1974). These techniques often include 24 to 48 h cultures to obtain mitoses, Q-banding, which requires photographic karyotyping for analysis, or trypsin G-banding, which is also time consuming and usually does not provide good banding quality (Rowley, 1974). In order for chromosome analysis to make a meaningful contribution to diagnosis and treatment in hematologic disorders, both the quality and speed of bone marrow chromosome preparation must be improved.

We have developed a bone marrow chromosome protocol that produces high quality metaphase banding that may be analyzed as soon as 2 h after marrow aspiration. The key features of this protocol are immediate processing of the bone marrow aspirate, an initial incubation with colcemid, modification of the hypotonic swelling treatment to include trypsin, and the use of a Wright's stain-banding technique, which allows immediate, direct karyotyping at the microscope.

Materials and Methods

Chromosome Preparation (New Protocol). Leukemic bone marrow specimens for chromosome studies are routinely obtained from an iliac crest aspirate. The specimen is collected in a heparinized syringe and processed as soon as possible, usually within 5 min after aspiration. This is best handled by attending the aspiration with the chromosome preparation solution in hand. Routinely, 0.2–0.5 ml of marrow aspirate is added to 10 ml of the following chromosome solution: nine parts 0.075 M KCl, one part 0.25% Trypsin-EDTA (Grand Island Biological Co. no. 610-5300), and colcemid (Grand Island Biological Co. no. 521) at a final concentration of 0.08 μg/ml. This cell suspension in chromosome solution is incubated at 37°C for 20 min, followed by centrifugation at 1200 rpm in an IEC clinical centrifuge for 5 min and fixation with 3:1 methanol:acetic acid for 20 min at

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room temperature. After two to three additional centrifugations and washing with fresh fixative, with intervening 5-min incubations at room temperature, slides are prepared using either the air-drying technique or by gentle warming of the slide over a gas burner. The best chromosome spreading is most often achieved with the warming method, and it causes no loss in the quality of banding.

**Chromosome Banding.** To achieve rapid banding of metaphase chromosomes, we have adopted the Wright's staining procedure of Sanchez et al. (1973) to replace the conventional G- and Q-banding techniques. This staining method does not require the usual slide ageing or pretreatment with trypsin used for Giemsa staining. It produces high contrast permanent banding, even on freshly made slides.

Slides are stained for 1.5–4 min in 2.5 mg/ml Wright’s stain (Matheson, Coleman, and Bell, Inc.) in methanol diluted 1:3 in 0.06 M NaHPO4, 0.06 M KH2PO4 (pH 6.8), then rinsed with water and air dried. The exact staining time will vary with the age of the stain, room temperature, age of the slides, and method of slide preparation.

Understained slides may be stained for additional 15-s intervals, while overstained slides may be “destained” (Rowley et al., 1971) in a series of organic solvents (95% ethanol for 2 min; chloroform for 15 s; 95% ethanol containing 1% v/v 6 N HCl for 30 s; 100% methanol for 2 min) followed by restaining with Wright’s. In some cases, slides were stained with Giemsa for illustrative purposes before destaining and restaining with Wright’s.

**Chromosome Preparation (Short Term Culture Technique).** For comparison purposes, a portion of each bone marrow specimen was processed using a more conventional method in which 0.2–0.5 ml of marrow was added to 10 ml Dulbecco’s Modified Eagles Medium containing 15% fetal calf serum (FCS) and 0.08 μg/ml colcemid for 30–60 min at 37°C. These specimens were then centrifuged as above, treated with 0.075M KCl for 10 min at 37°C and further processed as described above for fixation, slide preparation and banding.

**Comparison and Protocol.** We will refer to the new procedure of simultaneous chromosome treatment with trypsin, hypotonic solution, and colcemid as the THC method and will refer to the conventional procedure of short term culture in serum, medium, and colcemid as the SMC method. We evaluated a series of 50 consecutive bone marrow specimens using the general criteria of chromosome length, adequate spreading, and the ability to elicit bands to compare the two chromosome preparation procedures.

**Results**

Comparison of methods for preparation of banded chromosomes from bone marrow for clinical purposes should take into consideration both quality of banding and speed of preparation. We will concern ourselves first with quality, and will analyze the speed of preparation and its importance in clinical evaluation of chromosomes in the Discussion.

Fig. 1 shows the typical level of difference between the SMC and THC procedures using the same bone marrow specimen. These two preparations have been stained using Giemsa rather than Wright’s stain to show overall chromosome morphology without banding. SMC chromosomes (Fig. 1A) were typically short, with chromatids separated. In many cases, chromosomes are very poorly spread in addition to being highly contracted. In the case illustrated in Fig. 1A, all attempts to produce banding failed (see Fig. 1B). Fig. 1C shows a THC chromosome preparation from the same case. Chromosomes are longer, with chromatids together. After destaining as described in the Methods section and restaining with Wright’s stain, clear chromosome banding is obtained (Fig. 1D).

We should emphasize that Fig. 1 illustrates typical chromosome preparations from the same case using the two different techniques. It is possible to find higher and lower quality metaphase chromosomes using each technique. However, the overall quality, especially in terms of the ability to produce bands, is superior for the THC preparation.

Table 1 summarizes the results comparing THC and SMC procedures for 50 cases which include various leukemias, lymphoma with possible bone marrow involvement, and two studies of congenital defects. In 74% of the cases, the THC protocol gave clearly superior results, while in 24% of the cases, results were similar for the two techniques. In only one case was the SMC preparation considered superior. It should be noted that chromosome bands were obtained in virtually all cases where the THC protocol was used. The proportion of specimens that we were unable to analyze (for 500 consecutive cases) using the two methods outlined above, plus 24-h cultures incorporating the THC protocol, was 15%. This included specimens from treated patients and bone marrow transplant patients for which few or no mitoses could be found, and inadequate bone marrow aspirates.

Figs. 2 and 3 illustrate the level of banding quality that is available using the THC protocol, including Wright’s banding, in the analysis of abnormal karyotypes from bone marrow in leukemias and congenital studies. The patient whose karyotype is shown in Fig. 2, a 1-month-old female with multiple congenital defects, required immediate analysis because of her unstable clinical condition. The THC bone marrow preparation allowed a determination of trisomy 13 in 4 h, rather than the 3–4 days required using standard stimulated peripheral blood lymphocyte techniques.

The patient whose bone marrow karyotype is shown in Fig. 3 had clinical features of both preleukemia and acute myelogenous leukemia (AML). The banded karyotype was analyzed within 8 h after receiving the specimen and revealed a chromosome 8 trisomy, an abnormality often seen in AML (Mitelman et al., 1976; Van den Berghe et al., 1978). This result, along with other hematological findings, was compatible with a diagnosis of AML over preleukemia or a benign anemia.