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COMMUNICATIONS

Inhibitory effect of phenylbutazone and oxyphenbutazone on DNA synthesis in normal human bone marrow cells *in vitro*

Treatment with the antipyretic analgesic drugs phenylbutazone (PBZ) and oxyphenbutazone (OPB), mainly for rheumatic and allied disorders, is well known to be associated with bone marrow depression which may give rise to aplastic anaemia (Bithell & Wintrobe, 1967; Committee on Safety of Medicines, 1973). The side effects are serious enough to warrant frequent monitoring of patients' blood counts during prolonged medication, though this is not universally observed. The nature and range of these blood dyscrasias suggests that the side effects may be brought about by some fundamental action similar to that of the cytotoxic drugs, many of which interfere with DNA or with DNA synthesis (Balis, 1968). Such interference can adversely affect cell production in several ways, including reduction in the numbers of proliferating cells, their rate of proliferation and increase of cell death rate after production.

Studies on the association of chromosomal damage and PBZ or OPB treatment have been equivocal. Some workers have shown increases in the incidence of chromosomal aberrations with PBZ in cultured lymphocytes from human patients treated *in vivo* (Stevenson, Bedford & others, 1971) and in cultured human lymphocytes from untreated subjects when PBZ was administered *in vitro* (Wissmüller, 1971). Other reports have shown no significant difference in PBZ-treated and control lymphocytes from human patients (Walker, Price Evans & others, 1975) and horses (Stevenson, Hastie & Archer, 1972) and also in bone marrow cells from other animals (Müller & Strasser, 1971; Jensen, 1972; Gebhart & Wissmüller, 1973). Wissmüller (1971) observed that PBZ at therapeutic concentrations could inhibit mitosis in cultures of human lymphocytes. A similar effect was found in Ehrlich ascites tumour cells cultured with PBZ continously for 48 h and also a small reduction in mean cellular DNA content was found after this treatment (Breull & Karzel, 1970).

The nucleoside thymidine is specifically incorporated into DNA in the DNA-synthetic phase of the cell cycle, and any reduction in the uptake of tritiated thymidine (^{3}H-TdR) in bone marrow cells caused by the drugs would infer that haematopoiesis in treated patients would be impaired and long term therapy could lead to the blood disorders described. We have therefore investigated the direct effect of PBZ and OPB *in vitro* on the rate of ^{3}H-TdR uptake by normal human bone marrow cells and report some results here.

Bone marrow cells were aspirated from healthy ribs removed at thoracotomy from patients with non-invasive bronchial carcinomas. Replicate cultures of 10^6 cells suspended in 1 ml Eagle's MEM with 5 μCi ^{3}H-TdR (spec. act. 52 Ci mM^{-1}) and different concentration of either PBZ or OPB were incubated for 1 h at 37°. The final concentrations of these drugs ranged from 10–200 μg ml^{-1} to cover the range of serum concentrations found in patients undergoing long-term PBZ or OPB therapy (Walker & others, 1975). Because both drugs bind so strongly to plasma proteins (94–99% according to Prescott, 1968), the effects of incubation with and without serum (20%) were compared. For uniformity, serum from a separate unmedicated person was used throughout these experiments. After stopping incorporation of ^{3}H-TdR

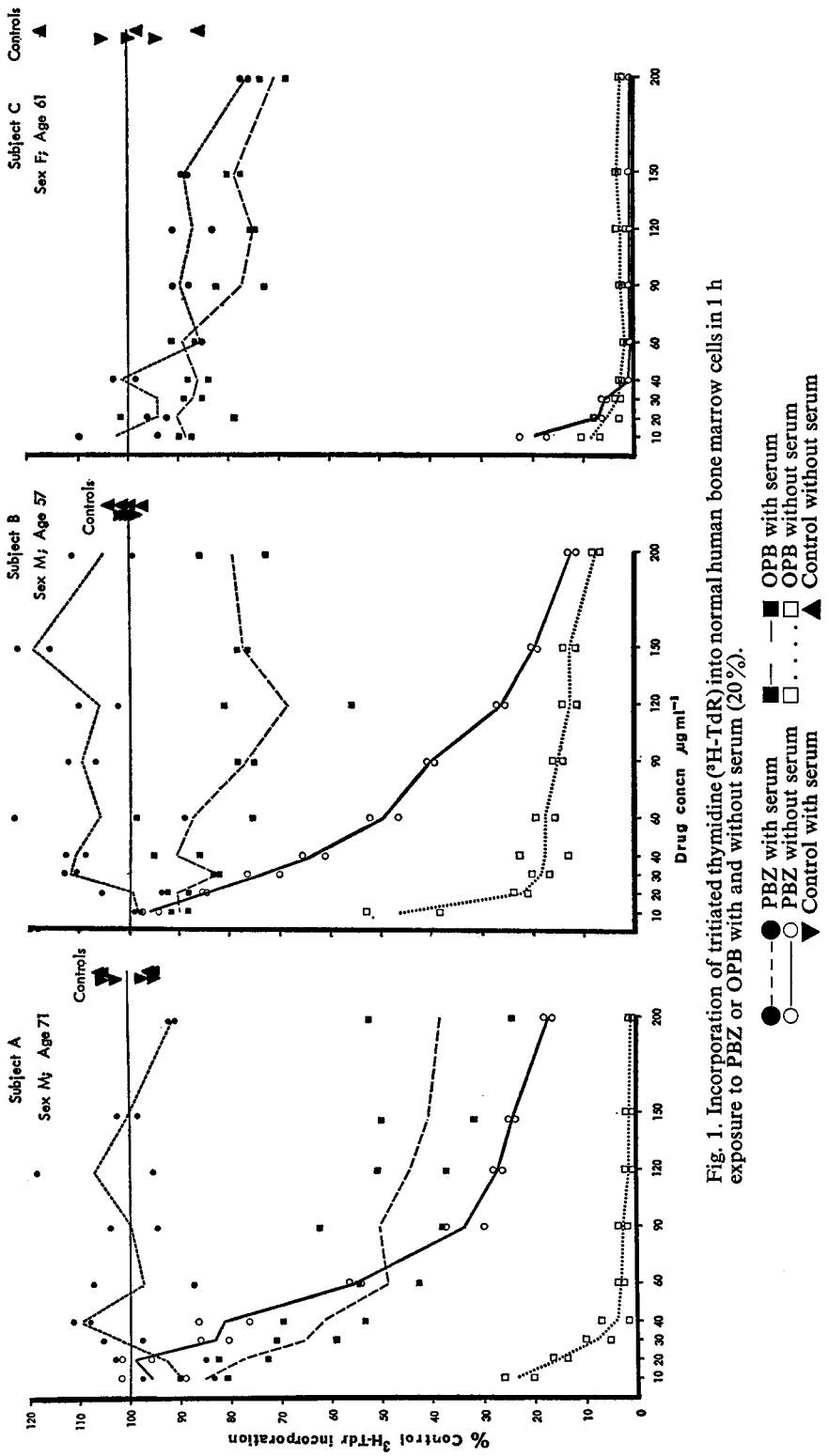

Fig. 1. Incorporation of tritiated thymidine (^{3}H-TdR) into normal human bone marrow cells in 1 h exposure to PBZ or OPB with and without serum (20%).

by the addition of 0·1 ml concentrated (150 mM) cold thymidine, the cells were collected on fibreglass discs in a Millepore sample manifold, washed with 5% TCA and methanol, and the ^{3}H-TdR incorporated was estimated using Aquasol (NEN) scintillation cocktail on a Packard Tri-Carb liquid scintillation spectrometer. In each case, the amount of ^{3}H-TdR uptake was represented as percentage of uptake in parallel cultures with no drug added (controls).

The graphs show data obtained with cells from three different subjects and illustrate the variation in the effects of the drugs on ^{3}H-TdR incorporation in bone marrow cells from different patients and the modification of these effects by addition of serum. The effects of PBZ without serum are similar in marrow from subjects A and B, where a gradual reduction of ^{3}H-TdR uptake is observed with increased concentrations, but in these cases serum completely negates the action of this drug. In C, however, the sensitivity of the cells to PBZ without serum is much greater than in A and B and the response shows a sharp threshold. The moderating action of serum is also reduced in C, so that there is still some inhibition of ^{3}H-TdR incorporation even with PBZ concentrations well within the therapeutic range. The inhibitory effect of OPB without serum is severe and shows a sharp threshold in each instance. In cases A and C, ^{3}H-TdR uptake is almost fully inhibited, though in A the effect of OPB is considerably greater than that of PBZ whereas in C the two drugs cause comparable depression of ^{3}H-TdR incorporation. In B, although a sharp threshold is again apparent, the inhibition is less complete. Addition of serum again moderates the inhibition by OPB, but in each case there is still a pronounced reduction in ^{3}H-TdR uptake at therapeutic drug concentrations. It will be noted that subject C whose cells showed the greatest response to the drugs was the only female in the group illustrated, but other data suggest that the sex of the subject is not an important factor in determining this response.

Cell counts on parallel cultures in these experiments showed cell numbers to be unchanged after incubation with each of the drugs over the range of concentrations used. Cell viability as shown by trypan blue exclusion showed a slight but statistically non-significant decrease with increasing concentrations of each drug, reducing by only some 6% at the higher drug concentrations used. Lethal toxic effects of the drugs in this short exposure can be discounted therefore, and the reduction in ^{3}H-TdR incorporation can be taken to reflect a real inhibition of DNA synthesis caused by the drugs. Thus we have found that short-term *in vitro* exposure of normal human bone marrow cells (and of mouse bone marrow cells in preliminary experiments) to therapeutic concentrations of both PBZ and OPB can cause variable and sometimes severe inhibition of DNA synthesis during that exposure. This suggests that long-term exposure to these drugs could cause continued (and possibly cumulative) reduction of haematopoiesis, which could produce the blood dyscrasias reported in some patients undergoing prolonged treatment.

From our observations we also conclude that bone marrow cells both from the same subject and from different subjects vary in sensitivity to each of the two drugs and in the degree of moderation of the effects of the drugs by serum, presumably by protein binding. The differences revealed in these experiments may be responsible for the different susceptibilities of patients to the side effects of these drugs, and this finding, which we are investigating further, may form the basis of a pre-treatment test to identify susceptible patients.

The response of the cells of subjects A and B to PBZ and its metabolite OPB when no serum was added could fit the interpretation of rate-limited metabolism of inactive PBZ to the active metabolite OPB which then causes inhibition of DNA synthesis. However, this biotransformation is not rapid *in vivo* (20% daily according to Woodbury, 1972), is normally a function of hepatic cells and has not been shown to occur in

bone marrow cells, so it is unlikely that this metabolism could completely account for the variability observed. Further, the different response by subject C's cells, where the inhibitory effect of PBZ was comparable to that of OPB, also suggests that there may be factors other than differential metabolism of PBZ to OPB involved.

It should be emphasized that this considerable inhibition of DNA synthesis in bone marrow cells has been brought about by only 1 h's exposure to therapeutic concentrations of these drugs, whereas many patients may be exposed to the drugs for a number of years. It is also important to note that the magnitude of the inhibition of DNA synthesis found to be caused by both these drugs without the addition of serum proteins, and by OPB even in the presence of serum, has been comparable to that caused by severely cytotoxic drugs used in chemotherapy of leukaemias, for example, cytosine arabinoside (Willmanns, 1971) and hydroxyurea (Potter, unpublished observations).

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Cytogenetics Unit
Nuffield Wing,
School of Medicine,
University, Liverpool, L69 3BX, U.K.

C. D. DEWSE
C. G. POTTER

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