Acknowledgements. We thank Cynthia L. Pouncey for taking the skinfold measurements and the volunteers for their enthusiastic cooperation. The research was supported in part by a grant from The Rowland Foundation of Cambridge, Massachusetts. The process used was patented by Louisiana State University. A. H. Meier and A. H. Cincotta have financial interest in the process.

* This is a process patented by Louisiana State University and licensed to Ergo, Inc., Newport, Rhode Island. A. H. Meier and A. H. Cincotta have financial interest in the process.

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Research Articles

Effect of dipyridamole and adenosine monophosphate on cell proliferation in the hemopoietic tissue of normal and gamma-irradiated mice

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Received 15 April 1991; accepted 30 September 1991

Abstract. Combined treatment with dipyridamole and adenosine monophosphate enhances cell proliferation in the hemopoietic tissue of normal and gamma-irradiated mice. This effect can be explained by the elevation of extracellular adenosine, and the receptor-mediated activation of the cell adenylate cyclase system.

Key words. Dipyridamole; adenosine monophosphate; cell proliferation; hemopoiesis; gamma-irradiation.

Adenosine, an endogenous purine nucleoside, is an important regulatory metabolite which exerts modulating effects on various physiological processes. Some in vitro studies indicate that adenosine could stimulate the proliferative activity of cells of the hemopoietic tissue. An increased spontaneous incorporation of 3H-thymidine into thymocytes after incubation with high doses of adenosine was reported, and the addition of adenosine or adenosine monophosphate to long-term bone marrow cultures resulted in an increased granulocyte produc-
The aim of the present work was to explore the possible role of adenosine in the regulation of cell proliferation in vivo, in the hemopoietic system of mice. The investigation of the role of extracellular adenosine was based on the evidence that the physiological effects of adenosine are primarily regulated through adenylate cyclase via specific cell surface receptors. However, the rapid decomposition of interstitial adenosine, and also its rapid uptake by cells, make this task difficult. In order to overcome this difficulty, dipyridamole, a drug which inhibits the cellular uptake of adenosine and thus increases its extracellular levels, was administered to mice together with adenosine monophosphate, a soluble adenosine prodrug which is metabolized extracellularly to adenosine by ecto-enzyme nucleotidase. This experimental regimen was expected to increase the level of extracellular adenosine and to induce observable effects on cell proliferation. Both the drugs were given either alone or in combination. The experiments were performed on normal mice and on mice in which cell proliferation was inhibited by gamma-irradiation.

Materials and methods
Hybrid male mice (CBA × C57BL/10)F1, three months old and with an average body weight of 30 g, were used. Standard stock diet and acidified tap water were given ad libitum. The mice were caged in groups of 20. Control and experimental procedures were carried out concurrently in groups of mice from the same cage. Dipyridamole (Sigma, USA) was dissolved in 0.4% tartaric acid and injected s.c. at a dose of 2 mg per mouse, in a volume of 0.4 ml. Adenosine 5'-monophosphate sodium salt from yeast (Sigma, USA) was dissolved in distilled water and injected i.p. at a dose of 5 mg base per mouse, in a volume of 0.2 ml. Dipyridamole (DP) and adenosine monophosphate (AMP) were given in single injections either alone or in combination, DP being administered 20 min before AMP (DP + AMP). Tartaric acid (0.4%) and saline were used for control s.c. and i.p. injections. The assay of cell proliferation was performed by measuring 125I-labeled 5-iodo-2'-deoxyuridine (125IUDR) incorporation into the DNA of the spleen and femoral marrow cells. The mice were given i.p. 7.4 × 10^4 Bq 125IUDR (Institute of Nuclear Research, Prague) in 0.4 ml saline. After 24 h the mice were killed and the spleens and left femurs were placed in 10% buffered formalin for 48 h to remove radioactive iodine not incorporated into DNA. The radioactivity of the organs was measured with a well-type counting system and expressed in cpm. Since there is evidence that 125IUDR incorporation into cells terminates up to 30 min after its administration, the data on cell proliferation relate to the time of IUDR injection. The proliferation activity in terms of 125IUDR incorporation was ascertained 24 h after the administration of drugs in normal unirradiated animals or 24 h after radiation exposure in irradiated and drug-treated mice. The mice were irradiated with single whole-body doses from a 60Co gamma-ray source, at a dose rate of 0.37 Gy/min. The time intervals between the administration of drugs and radiation exposure denote the time in minutes between the 2nd injection (AMP or saline) and the start of irradiation. Nucleated cells in the spleen and femoral marrow were determined by a Coulter Counter after washing off the marrow of femoral diaphyses, and crushing the spleen tissue. Hemopoietic progenitor cells committed to granulocyte-macrophage differentiation (GM-CFC) were assayed by a semi-solid plasma-clot technique. Briefly, bone marrow and spleen cell suspensions were plated in quadruplicate using 10% murine lung-conditioned medium as a source of colony-stimulating factor. Colonies (> 50 cells) were counted after 7 days of incubation in a 37°C humidified environment containing 5% CO2. For differential counting of the main cell lines, bone marrow and spleen smears were stained by the May-Grünewald and Giemsa-Romanowski method. Statistical significance of the results was evaluated using the t-test. The values given in the figure and tables represent the means ± SEM.

Results and discussion
As shown in the figure, the combined use of DP + AMP enhances cell proliferation (125IUDR assay) in the spleen of normal mice, as well as in those irradiated with a dose of 2 Gy 15 min after the administration of the drugs. The dose of 2 Gy decreases cell proliferation in control spleen organs.