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Hydroxyurea: Inhibition of DNA-Synthesis and Selective Susceptibility of S-Phase Cells *In Vivo* *

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Various proposals have been made to explain the mode of action of hydroxyurea, an agent known to inhibit experimental tumours in mice (Stearns *et al.*, 1963) and chronic granulocytic leukemia in man (Krakoff *et al.*, 1963; Thurman *et al.*, 1963). The agent degrades deoxyribonucleic acid (DNA) and this has been suggested to be the cause of chromosome fragmentation induced in cells that are exposed *in vitro* (Borenfreund, 1961). The same chemical and biological effects are produced by hydroxylamine and hydroxyamic acid congeners of hydroxyurea (Borenfreund, 1961; Somers and Hsu, 1961). Detection of acetohydroxamic acid in the blood of patients under treatment with hydroxyurea has suggested that the latter is split *in vivo* to hydroxylamine that in turn reacts with acetyl coenzyme A; a portion of the hydroxylamine released by hydrolysis might in theory degrade DNA and fragment chromosomes in susceptible cells (Fishbein and Carbone, 1963). Growth inhibition of cells in culture may be blocked to some extent by addition of pyrimidine deoxyribonucleosides but not by ribonucleosides (Mohler, 1964). This is consistent with the observation that treatment with hydroxyurea inhibits the reduction of cytidylic acid to the deoxyribonucleotide *in vivo* in rat bone marrow and in tumors in patients (Frenkel *et al.*, 1964). Finally hydroxyurea can induce prompt inhibition of DNA-synthesis in microorganisms (Rosenkranz and Levy, 1965; Gale *et al.*, 1964), in mammalian cells in culture (Young and Hodes, 1964; Sinclair, 1966; Gale, 1964; Yarbro *et al.*, 1965), and in regenerating liver in rats (Schwartz *et al.*, 1965) without interfering with the synthesis of ribonucleic acid or protein. The inhibition of DNA-synthesis is quickly reversible.

Hydroxyurea induces in rats karyorrhexis in germinal centers of all lymphoid tissues, bone marrow, and intestinal crypts within a few hours after injection (Philips *et al.*, 1964.) Acute cytotoxic effects are limited selectively to those tissues with

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high rates of proliferation. The pathologic process is of short duration and repair of tissue defects is rapid. Other hydroxamic acid derivatives induce similar lesions: N-hydroxyurethan, acetohydroxamic acid, N, O-diacetyl-N-methylhydroxylamine, and 1-methyl- and 1-ethyl-1-hydroxyurea. The lesions are not seen in rats given hydroxylamine or N-methylhydroxylamine.

The cytotoxic effects are related in time to the physiological disposition of hydroxyurea. The agent is rapidly equilibrated throughout the body water. Its concentration in plasma decays exponentially with half-lives of 65 minutes at levels above 150 μg/ml and of 35 minutes, below 100 μg/ml. N-hydroxyurethan and acetohydroxamic acid are disposed of *in vivo* at comparable rates. All these substances are excreted unchanged in urine and also extensively metabolized. The close temporal relation between the build-up and repair of cytotoxic changes and the physiological disposition suggests that the proximal biochemical defect produced by hydroxamates is reversible.

Hydroxyurea induces an immediate inhibition of thymidine (TdR) incorporation into the DNA of thymus and small intestine in rats. The inhibition is reversible; its duration varies directly with dose of hydroxyurea. From information about tissue concentrations it can be concluded that the sensitibity of TdR incorporation in intestine and thymus *in vivo* is close to that of HeLa cells in culture (Young and Hodes, 1964). The cytotoxic potency of hydroxyurea, N-hydroxyurethan, and acetohydroxamic acid in intact rats is in proportion to their relative activity in inhibiting TdR incorporation in Hela cells (Young and Hodes, 1964).

Autoradiographic studies of the incorporation of TdR-H^3 into crypt epithelium of mouse intestine show that lethal susceptibility to hydroxyurea is restricted to those cells which are synthesizing DNA (that is, cells in the S-phase of the mitotic cycle). Cells in other phases of the mitotic cycle (G_1, G_2, and M) during the limited time of circulation of inhibitory concentrations of hydroxyurea escape damage. Presumably such a mechanism accounts both for the wave of cytotoxic changes which follow injection of hydroxyurea and for the prompt repair of tissue defects.

The absence of a prolonged state of injury in proliferative tissues differentiates the action of hydroxyurea and its congeners from that of other antitumor agents. It is, for example, well-known that x-irradiation can induce long periods of mitotic arrest that are usually followed by the appearance of abortive mitoses and atrophy (Patt and Quastler, 1963). These are equally well-known effects of most cancer chemotherapeutic agents. Nevertheless, if hydroxyurea-like compounds are given repetitively, it is possible to induce significant depletion of normal proliferative tissues and to inhibit tumors (Stearns *et al.*, 1963; Krakoff *et al.*, 1963; Thurman *et al.*, 1963; Adamson *et al.*, 1964; Adamson, 1965; Rosenthal *et al.*, 1928.) Presumably each successive dose in a series will kill that fraction of each proliferative compartment which enters into DNA-synthesis during each recovery period. With appropriate spacing of doses cumulative reductions of proliferative compartments will ensue. Whether optimal dose schedules can be designed to accomplish maximal destruction of tumor cell compartments without undue depletion of normal proliferative tissues is worthy of some consideration.

Summary

The cytotoxic effects of hydroxyurea and of related hydroxamic acid derivatives *in vivo* are briefly described. They occur selectively in tissues with high rates of cell renewal and they are of brief duration. Tissue concentration of hydroxyurea diminish rapidly as the result of renal excretion and metabolism; there is a close temporal relation between the physiological disposition of the agents and the cytotoxic changes. Hydroxyurea induces an immediate inhibition of DNA synthesis in proliferating tisdsues such as thymus, small intestine, and regenerating liver. Autoradiographic studies of mouse duodenum using tritiated thymidine have shown that the lethal susceptibility to hydroxyurea is restricted to cells in the S-phase of the mitotic cycles. Cells in G_1, G_2, and M are not damaged by the agent.

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RIASSUNTO

Vengono brevemente descritti gli effetti citostatici *in vivo* dell'idrossiurea e dei derivati dell'acido idrossamico. Questi si verificano selettivamente in tessuti ad elevato tasso di rinnovo cellulare, e sono di breve durata. La concentrazione tissutale di idrossiurea diminuisce rapidamente in conseguenza dell'escrezione renale e del metabolismo; esiste uno stretto rapporto temporale fra il metabolismo degli agenti ed i cambiamenti citotossici. L'idrossiurea induce un'immediata inibizione della sintesi dell'ADN in tessuti proliferanti, quali timo, intestino tenue e fegato in rigenerazione. Studi autoradiografici del duodeno nel topo, mediante timidina tritiata, hanno dimostrato che la suscettibilità letale all'idrossiurea è limitata alle cellule nella fase S dei cicli mitotici. Le cellule in G_1, G_2 ed M non vengono danneggiate.

RÉSUMÉ

Les effets cytotoxiques de l'hydroxyurée et des dérivés de l'acide hydroxamique *in vivo* sont brièvement décrits. Ils se vérifient sélectivement en des tissus avec un taux élevé de renouvellement cellulaire, et sont de brève durée. La concentration tissutale de l'hydroxyurée diminue rapidement en raison de l'excrétion rénale et du métabolisme; il existe un étroit rapport temporal entre métabolisme des agents et changements cytotoxiques. L'hydroxyurée induit une inhibition immédiate de la synthèse de l'ADN dans les tissus prolifératifs, tels que le thymus, l'intestin grêle et le foie régénératif. Des études autoradiographiques du duodène chez la souris, moyennant thymidine tritiée, ont démontré que la susceptibilité léthale à l'hydroxyurée est limitée aux cellules dans la phase S des cycles mitotiques, les cellules dans les phases G_1, G_2 et M n'étant pas endommagées.

ZUSAMMENFASSUNG

Kurzer Bericht über die zytostatische Wirkung *in vivo* der Hydroxyurea und der Hydroxaminsäure-Derivate. Die Wirkung zeigt sich vorwiegend in Geweben mit grosser Zellerneuerung und hält nur kurze Zeit an. Die Hydroxyurea-Konzentration im Gewebe nimmt durch Nierenausscheidung und Stoffwechsel rasch ab. Es besteht ein enger chronologischer Zusammenhang zwischen der physiologischen Disposition der Wirkstoffe und den zytotoxischen Veränderungen. Die Hydroxyurea bewirkt eine sofortige Inhibition der DNS-Synthese in den proliferativen Geweben wie Thymus, Dünndarm und regenerative Leber. Autoradiographische Untersuchungen beim Duodenum von Mäusen mittels Thymidina tritiata bewiesen, dass die Hydroxyurea nur für die Zellen letal werden kann, die sich in der S-Phase der Mitose befinden. Die in den Phasen G_1, G_2 und M befindlichen Zellen erleiden keinerlei Schaden.

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