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ACTION OF SOME DRUGS ON ENZYMES INVOLVED IN DNA-REPAIR AND SEMICONSERVATIVE DNA-SYNTHESIS

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ABSTRACT

Different antirheumatic and cytostatic drugs had been tested by measurement of the thymidine incorporation into DNA of spleen cells under conditions, under which either DNA-synthesis or repair after Gamma- or UV-irradiation takes place. There are substances, which inhibit either only the semiconservative DNA-synthesis (Vinblastine, Isonicotinic acid hydracide) or only DNA-repair after Gamma-irradiation (mixture of Penicillin-G and Procaine-Penicillin-G) or both (Cyclophosphamide, Phenylbutazone, Procarbazine, Nalidixic acid).
Vincristine shows no effect on the thymidine incorporation in DNA, but by density gradient centrifugation it has been found that it influences the ligase reaction.
Two DNA polymerases had been isolated from spleen cells, one of the low molecular and one of the high molecular weight type.The influences of the described drugs on these enzymes and on a Deoxyribonuclease I from beef pancreas have been tested in "in vitro" systems. In all cases, it has been found that there is no effect or only a very small one, compared with the action of well known inhibitors as e.g. Ethidium bromide and p-Chloromercuribenzoate, and this cannot be responsible for the suppressions found in DNA-repair and semiconservative DNA-synthesis.

Key Words: DNA-SYNTHESIS/DNA-REPAIR/SPLEEN CELLS/DNA-POLYMERASE/DNASE/DRUGS/ CM

ÜBER DIE WIRKUNG EINIGER PHARMAKA AUF ENZYME DER DNA-REPARATUR UND DER SEMIKONSERVATIVEN DNA-SYNTHESE

KURZFASSUNG

Verschiedene Antirheumatika und Zytostatika wurden durch

Messung des Thymidineinbaues in die DNA von Milzzellen sowohl auf ihren Einfluß auf die semikonservative DNA-Synthese als auch auf die DNA-Reparatur nach Gamma- bzw. UV-Bestrahlung untersucht. Von den untersuchten Substanzen hemmten Vinblastinsulfat und Isonikotinsäurehydrazid die semikonservative DNA-Synthese, eine Mischung von Penicillin-G und Procain-Penicillin-G die DNA-Reparatur und Cyclophosphamid, Phenylbutazon, Procarbazin und Nalidixinsäure beide Synthesearten. Vincristinsulfat zeigt keinen Effekt auf den Thymidineinbau in die DNA. Allerdings wird durch diese Substanz die mit Hilfe der Gradientenzentrifugation in alkalischer Saccharose untersuchte Strangheilung von Einzelstrangbrüchen der DNA verzögert.
Zwei DNA-Polymerasen - eine niedermolekulare und eine hoch molekulare Polymerase - wurden aus Milzzellen isoliert. Der Einfluß der erwähnten Pharmaka auf diese beiden Enzyme sowie auf eine aus Rinderpankreas isolierte Desoxyribonuklease 1 wurde in "in vitro" Systemen untersucht. Dabei zeigten sich in allen Untersuchungen im Vergleich zu bekannten Inhibitoren, wie Ethidiumbromid und p-Chloromercuribenzoat, keine bzw. nur geringe Effekte. Die deutliche Unterdrückung, wie sie bei der Messung der DNA-Reparatur und der semikonservativen DNA-Synthese teilweise gefunden wurde, kann daher nicht in einer spezifischen Wirkung auf eines der drei Enzyme begründet sein.

Deskriptoren: DNA-SYNTHESE/DNA-REPARATUR/MILZZELLEN/DNA-POLYMERASE/DNASE/PHARMAKA/

ACTION OF SOME DRUGS ON ENZYMES INVOLVED IN DNA-REPAIR AND SEMICONSERVATIVE DNA-SYNTHESIS

In order to make investigations of DNA-repair systems on an enzymatic level, we took some drugs, which were by previous experiments (1,2) proved to be inhibitors of the DNA-metabolism. We tested these drugs for possible inhibitory effects on purified enzymes in cell-free systems.
The substances we used were partly antineoplastic and anti-tumor agents, some others produce side effects (3,4,5), which would indicate a possible influence on the genetic material (all listed in table 1).

TABLE 1: List of tested drugs and their properties

Drug	Medical use	side effects
VINBLASTINE	antineoplastic	chromosome aberrations
VINCRISTINE	antineoplastic	chromosome aberrations
CYCLOPHOSPHAMIDE	alkylating antineoplastic	chromosome aberrations
PROCARBAZINE	antineoplastic	
PENICILLIN-G/ PROCAINE-PENICILLIN-G	antimicrobial	
PHENYLBUTAZONE	analgesic, anti-pyretic, anti-rheumatic	chromosome aberrations
ISONIAZID	tuberculostatic	carcinogen, photosensitization of skin
NALIDIXIC ACID	antibacterial	photosensitization of skin

METHODS

DNA-repair and semiconservative DNA-synthesis:

Immediately after killing the mice, the spleen had been taken and homogenized, the washed cells were preincubated in HANKS with the drug and irradiated with ^{60}Co-Gamma-rays (30krad) or 254 nm UV-light (7500 erg/mm^2) at zero degree centigrades. Tritiated thymidine had been added (5 µCi/ml) and the incorporation of the activity into the DNA after different incubation times was stopped by addition of perchloric acid, the sediment was hydrolysed at 90°C and aliquots of the supernatant were measured for activity and DNA-content (6). The tested drugs were used in concentrations close to the level that will appear in blood after medical treatment (Vincristine 2,5 µg, Vinblastine 2,0 µg, Cyclophosphamide 50 µg, Penicillin-G/ Procaine-Penicillin-G 750 µg and all others 100 µg/ml).

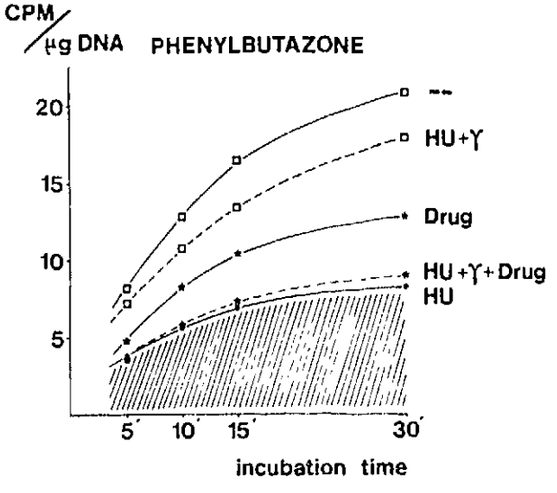

FIGURE: Control, non irradiated (□ —— □), sample with HU, irradiated (□ --- □), sample with the applicated drug, non irradiated (* —— *), sample with the applicated drug and HU, irradiated (* --- *), sample with HU, non irradiated (● —— ●).

$$\text{Inhibition of synthesis} = \frac{(\text{Drug}) - (\text{HU})}{(\text{——}) - (\text{HU})} \times 100$$

$$\text{Inhibition of repair} = \frac{(\text{HU+}\gamma\text{+Drug}) - (\text{HU})}{(\text{HU+}\gamma) - (\text{HU})} \times 100$$

In order to distinguish between semiconservative- and repair-synthesis, hydroxyurea (HU, 10^{-2}M) had been used. By combination of irradiated and unirradiated samples with or without HU and with and without the substance to be tested it is possible to get informations about influences on semi-conservative synthesis and repair, so far as endonuclease, exonuclease and DNA-polymerase are concerned, but not for the ligase reaction.

DNA-polymerase:

The activity of the DNA-polymerase was measured according to Wintersberger et al.(7) by mixing the enzymes with so called "activated DNA" - DNA nicked by short treatment with DNase I - for template and primer, and with the four necessary deoxy-nucleotidetriphosphates, buffer, Mg^{++}-ions and the inhibitor to be tested. One of the four triphosphates is ^{3}H-labeled and the incorporated activity was measured after 15 minutes incubation at 37°C.

DNase:

^{3}H-labeled DNA was mixed with enzyme and inhibitors and incubated at 37°C. The activity of the acid soluble fragments produced was measured (8).

In both assays for enzyme-activity all concentrations of the drugs were 100 µg/ml.

Metabolization of drugs:

Microsomes from rat liver were isolated and 1 mg of the drug was incubated with the amount of supernatant including microsomes that equals 0,25g of original liver at 37°C for one hour and then centrifuged at 100 000 x g (9).

RESULTS

The influence of the drugs on DNA-metabolism is indicated in TABLE 2. As there was no effect on DNA-repair after gamma-irradiation with most of the drugs, we metabolized some substances by mixing and incubating them with liver microsomes and found some inhibition as indicated by "m" in TABLE 2. There is no inhibition of the semiconservative DNA-synthesis by Cyclophosphamide, but sure there would be some effect by the metabolized drug. Ethidiumbromide we used for an inhibitor-

standard, because it is known to be a very strong inhibitor by complexing to the DNA. Those estimations of repair only include DNA-repair-synthesis, i.e. the nuclease and the polymerase systems and as we wanted to see the ligase too, we had to investigate the rejoining by sedimentation analysis in alkaline sucrose according to (2) as we did in some cases.

TABLE 2: Inhibition of semiconservative DNA-synthesis and DNA-repair-synthesis (Control=100, m= Drug metabolized, n.t.= non tested, significant deviation is indicated by fat numbers).

	Semiconservative DNA synthesis	Repair γ-irradiation	Repair UV-irradiation
VINBLASTINE	**66**	98/102 m	97
VINCRISTINE	106	102/106 m	95
CYCLOPHOSPHAMIDE	101	98/**79** m	98
PROCARBAZINE	**33**	101/**81** m	97
PENICILLIN-G / PROCAINE-PENICILLIN-G	94	**43**	n.t.
PHENYLBUTAZONE	**39**	**11**	n.t.
ISONIAZID	**22**	110	n.t.
NALIDIXIC ACID	**79**	**66**	**74**
ETHIDIUM BROMIDE	**6**	**0**	**13**

In TABLE 3 the inhibition of "repair-synthesis" and rejoining is compared. With Vincristine, there is some remarkable difference and there is possibly some inhibition of the ligase step.

TABLE 3: Inhibition of DNA-repair following either the kinetics of thymidine incorporation into DNA or the rejoining of strand breaks. All investigations were carried out with metabolized drugs for 30 minutes at 37°C.

30' repair after γ-irradiation

	% of control	
	by T-incorporation	by density-gradient
VINBLASTINE (m)	**102**	**100**
VINCRISTINE (m)	**106**	**60**
CYCLOPHOSPHAMIDE (m)	**79**	**70**
PROCARBAZINE (m)	**81**	**40**

For the "in vitro" experiments we used DNA-polymerase extracted and purified before from pig spleen. As in all mammalian cells, there are two types of this enzyme, one with high molecular weight (we call it the large polymerase) and another with low molecular weight (the small polymerase) (10). There are some evidences, that these enzymes play different roles in the cell, so remains the concentration of the "small" always constant, but the large one is very much stimulated in proliferating cells, regenerating tissue and tumors. The tested drugs did not inhibit these enzymes (TABLE 4) except a very small effect with metabolized Nalidixic-acid. Strong inhibition was observed with Ethidium-bromide (because of its reaction with DNA) and p-Chloromercuribenzoate (this reacts with the -SH-groups of the enzyme).

TABLE 4: Inhibition of small and large DNA-polymerase by unmetabolized and metabolized (met.) drugs. Control = 100.

	Inhibition of DNA polymerase			
	small		large	
	–	met.	–	met.
VINBLASTINE	119	116	104	113
VINCRISTINE	101	102	111	100
CYCLOPHOSPHAMIDE	108	116	112	111
PROCARBAZINE	112	109	97	100
PENICILLIN-G / PROCAINE-PENICILLIN-G	95	104	98	104
PHENYLBUTAZONE	109	112	100	103
ISONIAZID	112	99	93	101
NALIDIXIC ACID	109	**85**	92	**82**
ETHIDIUM BROMIDE	**3**	**18**	**2**	**5**
p-CHLOROMERCURIBENZOATE	**8**	n.t.	**<1**	n.t.

Similar experiments we did with DNase. We used DNase 1 from beef pancreas (Sigma chemicals) and this enzyme is not necessarily involved in the repair-system. We used it in order to try only if the type of reaction is sensitive to our drugs and also to discriminate effects on the polymerase from unspecific effects to the DNA. In future we will do experiments with more specialized nucleases too.

Only a small inhibition is produced by Nalidixic-acid (TABLE 5), but there is more distinct effect, if this drug is metabolized. There is also some influence with metabolized Cyclophosphamide and the inhibition by Ethidium-bromide is always total.

TABLE 5: Inhibition of DNase I (Control = 100).

	Inhibition of DNase	
	unmetabolized	metabolized
VINBLASTINE	100	110
VINCRISTINE	100	95
CYCLOPHOSPHAMIDE	92	80
PROCARBAZINE	90	101
PENICILLIN-G / PROCAINE-PENICILLIN-G	94	93
PHENYLBUTAZONE	90	105
ISONIAZID	98	104
NALIDIXIC ACID	78	30
ETHIDIUM BROMIDE	<2	<2

DISCUSSION

Our intention had been to find some specific inhibitors for the different enzymes. In TABLE 6 there are summarized the effects of the tested drugs on semiconservative DNA-synthesis, DNA-repair after gamma-irradiation, small and large DNA-polymerase and DNase. There are no correlations of the effects in the cellular systems with the free enzymes in the most cases. Thus we can say that the observed inhibition of the DNA-metabolism did not result from DNA-polymerase inhibition and also not from effects on DNases as far as our DNase I simulate any repair-involved endonuclease. Nalidixic acid shows some correlations, the polymerase inhibitions (or maybe only the inhibition of the large one) correlates with the DNA-synthesis and all enzymes together correlate with the more expressed effect on DNA-repair.
We needed metabolized Nalidixic-acid in order to influence the enzymes, but in the cellular systems, the cells were

able at least partly to metabolize Nalidixic-acid.
The effect of Ethidium-bromide is very strong, but not specialized enough to give informations on interactions of the enzyme-systems. Further experiments will show the influence of different drugs on more and specialized enzymes, i.e. different nucleases and ligase.

TABLE 6: Summary of results (* slight inhibition, ** strong inhibition, *** very strong inhibition). Investigations on semiconservative DNA-synthesis and DNA-repair were done with spleen cells, the measurements on the isolated enzymes in cell-free systems.

	Semiconservative DNA synthesis	γ-repair	DNA polymerase small	DNA polymerase large	DNase
VINBLASTINE	**	-	-	-	-
VINCRISTINE	-	-	-	-	-
CYCLOPHOSPHAMIDE	-	*(m)	-	-	*(m)
PROCARBAZINE	**	*(m)	-	-	-
PENICILLIN-G / PROCAINE-PENICILLIN-G	-	**	-	-	-
PHENYLBUTAZONE	**	***	-	-	-
ISONIAZID	**	-	-	-	-
NALIDIXIC ACID	*	**	*(m)	*(m)	*/**(m)
ETHIDIUM BROMIDE	***	***	***	***	***

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