The Effect of Caffeine on the Survival of u.v.-irradiated Herpes Simplex Type 1 Virus

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SUMMARY

Caffeine reduced the survival of u.v.-irradiated herpes simplex type 1 virus. The relative reduction of plaque count by caffeine was most pronounced in the second component of the survival curve. With more heavily irradiated virus, when multiplicity reactivation apparently played a major role, the caffeine effect was less marked.

The inactivation curve of u.v.-irradiated herpes simplex virus (HSV) is modified by the host cell repair mechanisms (HCR) (Lytle, 1971, 1972; Lytle, Aaronson & Harvey, 1972) and by multiplicity reactivation (MR) (Ross, Cameron & Wildy, 1972; Roubal & Vonka, 1973). The effect of caffeine, which is known to hamper HCR (Witkin, 1969), on the survival of u.v.-irradiated herpesviruses has been described by Zavadorova & Zavada (1968) for pseudorabies virus and by Lytle (1972) and Ross et al. (1972) for herpes simplex virus. In this paper we report the effect of this drug on the survival of irradiated HSV under conditions in which MR takes place.

The cell cultures, the virus, its irradiation and the assay techniques were the same as in the previous experiments (Roubal & Vonka, 1973). Caffeine was added to the first agar overlay at the concentration 0·5 mg/ml. The second overlay, containing neutral red but free of caffeine was added on the third day of incubation. The plaques were counted on the 8th day of incubation. At the concentration used the drug was without any apparent deleterious effect on the cultures; however, the unirradiated virus titre was slightly lower (by an average of 29 %) and the plaques were smaller (by about 50 %) and less sharply delineated than in the absence of caffeine.

The survival curves of u.v.-irradiated HSV are shown in Fig. 1. It can be seen that a lesser amount of irradiated virus survived in the presence of caffeine than when the drug was absent; however, in both the presence and absence of the drug the curves possessed multi-component character. In addition, in cultures inoculated with virus irradiated for 300 s or more, and kept either in the presence or absence of caffeine, non-linear relationship between the plaque count and virus dilution, indicative for MR, was observed.

In agreement with the results obtained by Lytle (1972) and by Ross et al. (1972) the present data demonstrate that caffeine has an inhibitory effect on the survival of u.v.-irradiated HSV, most probably due to the impairment of HCR. Still the survival in the presence of caffeine greatly differed from the theoretically expected values calculated from D37 dose for the first component of the survival curve. This may indicate that two different HCR mechanisms, one inhibited by caffeine and the other caffeine independent, are involved in the repair of the damaged virus genomes as suggested by Lytle (1972). However, it is also possible that the incompleteness of the effect was due to the involvement of some non-specific factors, such as the dependence of penetrability to the drug on the physiological state of the individual cells.

In the present experiments the relative reduction of the plaque count by caffeine was
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Fig. 1. Survival of u.v.-irradiated herpes simplex virus in presence and absence of caffeine. Each point represents a mean value from two separate experiments. Expressed as percentage of respective untreated virus titre. •—•, without caffeine; ○—○, with caffeine.

Fig. 2. Relative survival of u.v.-irradiated herpes simplex virus in presence of caffeine expressed as percentage of virus survival in absence of caffeine. Each point represents a mean value from two separate experiments.

differently expressed with different u.v. doses. The relative virus survival in the presence of caffeine expressed as the percentage of the virus survival in the absence of the drug is shown in Fig. 2. It can be seen that the relative plaque count in the presence of caffeine rapidly decreased with the dose of irradiation. The caffeine effect was at maximum at 120 to 180 s dose. This is in agreement with the findings of Lytle (1972) that the caffeine response is most pronounced in the second component of the survival curve. With the more heavily irradiated virus, the caffeine effect was less marked. With the increasing multiplicity of infection, MR apparently played a progressively increasing role and this could be responsible for the gradual reduction of the caffeine effect. Since, however, the suppressive effect of the drug was quite strong even in this phase of inactivation curve it seems that, within the limits tested, the removal of the u.v.-induced damages from the virus DNA was needed for effective
recombination in most instances; in other words, in the absence of the drug MR probably predominantly occurred between partially repaired virus genomes.

We were unable to measure reliably the plaque size in the presence of the drug caffeine, because the plaques formed in the presence of the drug were turbid. It may be of interest, however, that the curves describing the dependence of the caffeine effect (Fig. 2) and of the plaque size reduction on u.v. dose (Roubal & Vonka, 1973) possess similar shapes until 180 s dose. This and the observation that the small-plaque effect can be reversed by photoreactivation (Ross et al. 1972) might indicate that the caffeine sensitive step is involved in the removal of pyrimidine dimers the presence of which in the virus DNA could account for the delay in its replication (Ross et al. 1972).

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Department of Experimental Virology
Institute of Sera and Vaccines
Prague, Czechoslovakia

REFERENCES


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