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SIMULTANEOUS CISPLATIN AND RADIATION IN ENDOMETRIAL ADENOCARCINOMA CELL LINES

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The effects of concomitantly administered cisplatin and radiation were evaluated in four recently established endometrial adenocarcinoma cell lines. We used the 96-well clonogenic assay to obtain survival data which were fitted to the linear quadratic model. The area under the survival curve (AUC) was obtained by numerical integration. It turned out that there was only a systematic additive cytotoxic effect and no supra-additive, true radiosensitising effect could be found. The results were not affected by the cisplatin dose used, the intrinsic radiosensitivity of the cell lines or the sensitivity of the cells to cisplatin.

Simultaneous radiotherapy and chemotherapy is a widely studied modality to treat locally advanced solid tumors. The aim of this kind of therapy is to overcome radioresistance, which is the cause of local treatment failure, and to eradicate distant micrometastases, which are the cause of systemic spread. The antiproliferative effects of a combination of cisplatin and radiation have been studied in the clinical and experimental settings (1, 2). Advanced or recurrent endometrial carcinoma is usually treated with radiation and cisplatin-based chemotherapy. The concomitant use of these therapies in this tumour type has not been evaluated.

Clinical studies on combined chemotherapy and irradiation in squamous cell carcinoma (SCC) of the uterine cervix imply that it is feasible to use radiation and cisplatin simultaneously, but the therapeutic benefit is not clear (3–5). The toxicity, especially non-hematological, has in most cases been acceptable and comparable to radiation therapy alone, and this applies to both acute and late toxicity (6). However, a recent study suggests a higher late gastrointestinal complication frequency (5). Concomitant cisplatin and irradiation in adenocarcinomas has been studied to a less extent. A small study has been reported on this treatment modality for advanced colorectal adenocarcinoma (7).

In vitro chemoradiation experiments involving cisplatin have generally used cell lines of non-human origin (1) and there is not much data on the concomitant administration of cisplatin and radiation in human cancer cells (8–12). To our knowledge, only one study of this type has used endometrial carcinoma cell lines (13). The sensitivity of endometrial carcinoma to cisplatin varies greatly in vitro, up to 25-fold (14, 15). Furthermore, we have previously shown that endometrial cancer is as a group inherently radiosensitive compared to squamous cell carcinomas in vitro, but that there are significant differences in this respect between various cell lines (16).

Since the effects of cisplatin and radiation on endometrial carcinoma have been variable both clinically and in vitro, one may ask, if there is a synergistic or merely an additive interaction between cisplatin and radiation (17). The purpose of the present study was to evaluate the effects of simultaneous administration of cisplatin and radiation on four endometrial adenocarcinoma cell lines.

Material and Methods

Cell lines and cell culture. Four endometrial adenocarcinoma cell lines were tested. Two cell lines (UM-EC-1, UM-EC-3) have been established at the University of

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Table 1

Cell lines, their histologic type and grade, plating efficiencies (PE), their intrinsic radiosensitivities expressed as area under the survival curve (AUC) and α and β parameters of the LQ-model, their sensitivies to cisplatin expressed as IC_{50} values (50% inhibition of clonogenic survival) and references

Cell line	Histologic type and grade	Plating efficiency (PE)	Intrinsic radiosensitivity (AUC) (Gy)	α (Gy^{-1})	β (Gy^{-2})	Sensitivity to cisplatin (IC_{50}) ($\mu g\ ml^{-1}$)[c]	Reference
UM-EC-1	Adenocarcinoma, 3	0.41–0.46	1.75[b]	0.43	0.035	0.31	(18)
UM-EC-3	Papillary adenocarcinoma with focal clear cell change, 2	0.22–0.29	1.00[b]	0.82	0.081	0.13	[a]
UT-EC-2B	Adenocarcinoma with squamous metaplasia, 2	0.35–0.59	0.60[d]	1.8	−0.10	0.56	[d]
UT-EC-3	Adenocarcinoma, 3	0.32–0.44	1.81[c]	0.49	0.045	0.034	(19)

[a] Being characterized (S. Grénman)
[b] (16)
[c] (15)
[d] unpublished data
[e] (19)

Michigan, and two (UT-EC-2B, UT-EC-3) at the University of Turku by one of us (S.G.). The cell lines have been chosen on the basis of their dissimilar intrinsic radiosensitivity and sensitivity to cisplatin (15, 16). The biological characteristics of the cell lines are listed in Table 1. Three cell lines were established from primary tumours before any treatment. The UT-EC-2B cell line was established from a supraclavicular metastasis of an endometrial carcinoma in the patient diagnosed 17 months previously. The patient had received radiotherapy to the site of the primary tumour and six courses of combined chemotherapy containing cisplatin and hormonal therapy with medroxyprogesterone acetate before the detection of the supraclavicular metastasis. All cell lines were used at relatively low passage numbers (16–42). Prior to the experiments the cells were maintained in logarithmic growth in T25 culture flasks by passing weekly in Dulbecco's modified Eagle's minimal essential medium (DMEM) containing 2 mM L-glutamine, 1% non-essential amino acids, 100 U/ml penicillin, 100 U/ml streptomycin, and 10% fetal bovine serum (FBS). Cells in midlogarithmic growth (40–60% confluency) were used for the experiments, and the cells were fed with fresh medium on the day before plating.

Drug preparation. Cisplatin (Platinol, a kind gift from Orion Corporation, Orion-Farmos, Turku, Finland) 0.5 $mg\ ml^{-1}$ was diluted with growth medium to obtain a stock solution of 100 $\mu m\ ml^{-1}$ and sterilised by passing through a 0.22 μm filter. Final cisplatin dilutions of 0.005–0.4 $\mu g\ ml^{-1}$ were used, and fresh stock solutions were made for each experiment.

Clonogenic assay. The 96-well plate clonogenic assay based on limiting dilutions was used as described (20). Two to three experiments including duplicate plates were performed for each cell line. The cells were harvested with trypsin-EDTA to obtain single-cell suspension, counted, and diluted in Ham's F-12 medium containing 15% FBS. With a cell suspension containing 4167 cells ml^{-1} and diluted in 25 ml growth medium, a cell count of two cells per well is achieved by applying 100 μl to each well. The same single-cell stock solution was used as a source of cells for the experiments on the effect of radiation alone, cisplatin alone or combined treatment. The number of cells plated per well is adjusted according to the plating efficiency (PE) of the cell line. After plating into the 96-well plate the cells were allowed to attach for 24 h at 37°C in an incubator with an atmosphere saturated with water vapour containing 5% carbon dioxide. To study the combined effects of cisplatin and radiation, the drug solutions diluted into 100 μl Ham's F-12 medium were added to the cell cultures 1 h before the irradiation, and 100 μl of medium without cisplatin was added to the control plates and to plates which were only irradiated. The cells were irradiated in the plates with 4 MeV photons, generated by a linear accelerator (Clinac 4/100; Varian, CA); the dose rate was 2 Gy min^{-1}. The radiation doses were 0.75, 1.25, 2.50 and 5.00 Gy, except in the case of the highly radiosensitive UT-EC-2B cell line, which was irradiated with 0.4, 0.75, 1.25 and 2.50 Gy. The drug solutions were allowed to remain in the plates for the whole incubation period. The plates were incubated for 4 weeks, after which the number of wells containing coherent, living colonies (a colony consisting of 32 cells or more) was counted under an inverted phase-contrast microscope.

Data analysis. The plating efficiency (PE) was calculated by the formula PE = −ln (number of negative wells/total number of wells)/number of cells plated per well (21). The individual fractional survival data points as a function of radiation dose were fitted to the linear quadratic equation $F = \exp[-(\alpha D + \beta D^2)]$. The area under the survival curve (AUC) values, corresponding to mean inactivation doses

($\bar{D}$), were calculated by numerical integration. The simultaneous effects of cisplatin and radiation were measured as the ratio between the AUC for cisplatin plus radiation, divided by the AUC for radiation alone. This AUC-ratio was compared with the surviving fraction (SF_C) after the indicated dose of cisplatin alone. If the AUC-ratio and SF_C did not differ significantly, there was no true radiosensitising, supra-additive effect.

Results

The cisplatin–radiation interaction was studied on four endometrial adenocarcinoma cell lines. The PE values of the cell lines are listed in Table 1.

Three cisplatin doses were selected for each cell line based on earlier results on cisplatin sensitivities which are presented in Table 1 (15). The results of the cisplatin–radiation interaction experiments are summarised in Table 2 and Figure 1. In all cases, an additive effect was seen corresponding well to the calculated effect of radiation and cisplatin at the given concentration, but no supra-additive effect was seen in these experiments. This finding was not affected by the cisplatin dose expressed in SF_C. The cisplatin concentrations alone caused a 10–60% inhibition of clonogenic survival. UT-EC-3 was the most sensitive to cisplatin, and UT-EC-2B the most resistant to cisplatin, but these differences did not affect the results with respect to synergy. The cell lines differed also in their intrinsic radiosensitivity: UT-EC-2B was highly radiosensitive, and UM-EC-1 and UT-EC-3 were radioresistant. As for cisplatin, the intrinsic radiosensitivity of the cell lines did not affect the results with respect to cisplatin–radiation synergy.

Table 2

Effects of cisplatin on clonogenic survival of four endometrial adenocarcinoma cell lines used as a single agent and concomitantly with radiation

Cell line	Cisplatin dose ($\mu g\ ml^{-1}$)	$SF_C \pm SD$[1]	$\frac{AUC_{C+R}}{AUC_R} \pm SD$[2]
UM-EC-1			
	0.05	0.90 ± 0.08	0.87 ± 0.03
	0.1	0.83 ± 0.08	0.76 ± 0.05
	0.15	0.64 ± 0.05	0.61 ± 0.03
UM-EC-3			
	0.02	0.89 ± 0.12	0.89 ± 0.08
	0.05	0.67 ± 0.04	0.65 ± 0.05
	0.08	0.40 ± 0.08	0.39 ± 0.05
UT-EC-2B			
	0.2	0.89 ± 0.04	0.92 ± 0.07
	0.3	0.72 ± 0.03	0.75 ± 0.02
	0.4	0.59 ± 0.11	0.61 ± 0.12
UT-EC-3	0.005	0.93 ± 0.03	0.90 ± 0.02
	0.01	0.87 ± 0.05	0.84 ± 0.06
	0.02	0.79 ± 0.06	0.77 ± 0.06

[1] Surviving fraction obtained using indicated doses of cisplatin as a single agent

[2] The ratio between the AUC of cultures incubated with different doses of cisplatin concomitantly with radiation, divided by the AUC after radiation alone

Discussion

In the present study we evaluated how cisplatin and radiation administered together effected the growth of four endometrial carcinoma cell lines which had dissimilar sensitivity to cisplatin and dissimilar intrinsic radiosensitivity. Our results indicate that there was no true radiosensitising effect, but merely additive effect. Combined chemoradiotherapy in the clinic is faced with problems of dosage, timing and the sequence of drug administration. The current data does not give any clear guidelines for planning optimal therapeutic programs. It has been suggested that cisplatin–radiation interaction is based on preradiation enhancement due to altered binding of platinum complexes to DNA during radiation (22), or on postradiation enhancement due to inhibition of DNA repair mechanisms (23). It has been speculated that postradiation enhancement be clinically the more important mechanism of the two (24).

Studies on cisplatin-radiation interaction in experimental tumour models have given variable results. The first evidence of an actual interaction between cisplatin and radiation came from Zak & Drobnik in 1971 (25). A true radiosensitising effect of cisplatin on tumour cells has been reported occasionally in some animal tumours in vivo (26–31) and in vitro (32). Some investigators, on the other hand, have not found any enhancement in vivo (33) or in vitro (8, 9). Nguyen et al. in 1993 studied the concomitant use of chemotherapeutic agents, including cisplatin, and radiation on endometrial cancer cell lines (13). Their results, using non-clonogenic ATP bioluminescence assay, indicated that the effect of cisplatin and radiotherapy was synergistic.

Dritschilo et al. reported in 1979 that cisplatin inhibits repair of sublethal and potentially lethal radiation damage in vitro (23). Later, cisplatin was found to inhibit sublethal radiation damage repair (SLDR) of human SCC line only when it was administered at the time of radiation (10). In addition, some investigators have reported inhibition of SLDR only in hypoxic tumors (32, 34), while still others have found the same effect also in euoxic tumours (26). These controversial results depend partly on different experimental settings, including the oxygen status of the cells and the timing of cisplatin administration. We have recently demonstrated that the capacity of SLDR in endometrial carcinoma cell lines is rather limited (19). This characteristic may explain the purely additive effect of radiation and cisplatin in these endometrial cancer cell lines.

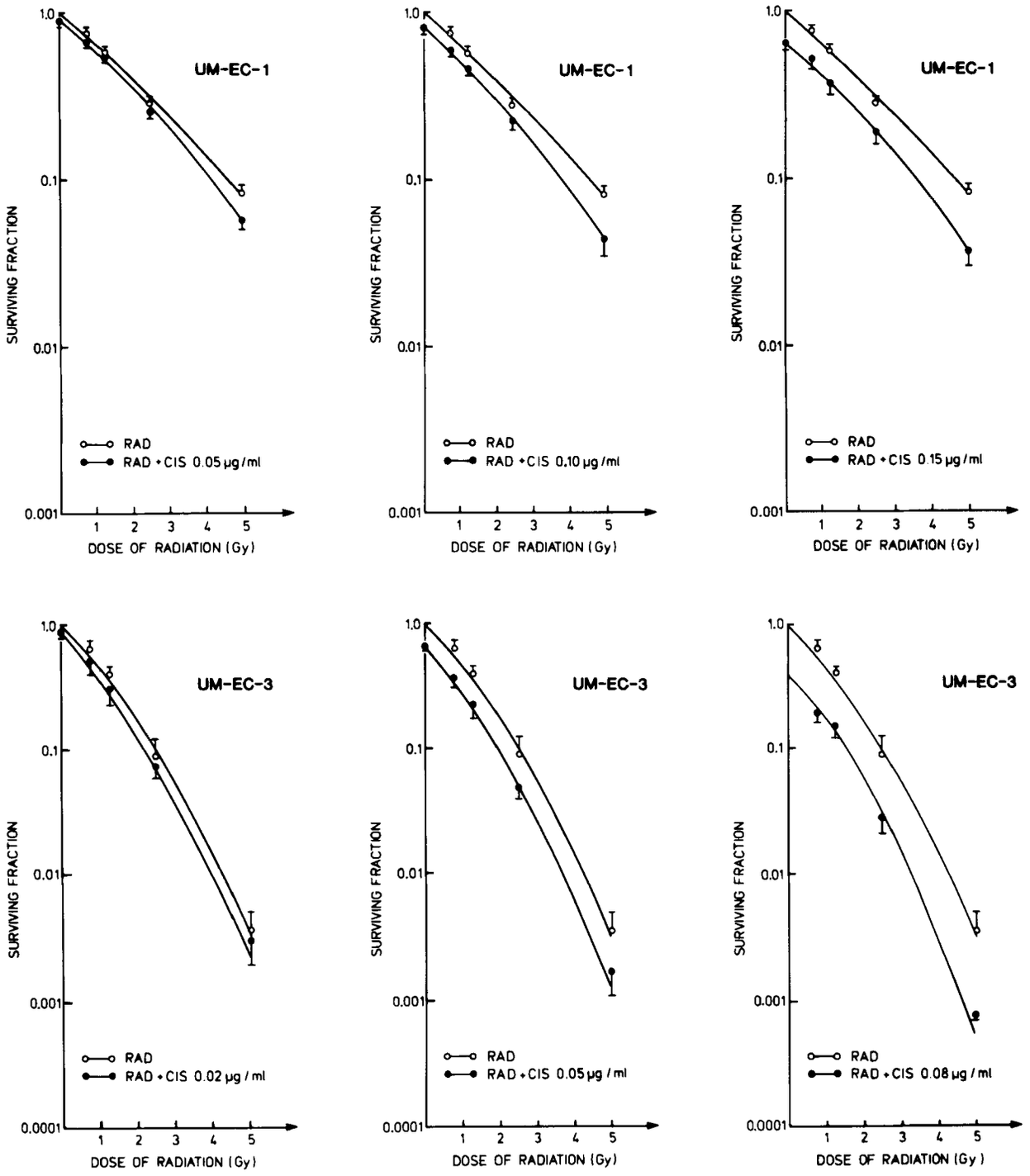

Fig. 1A. Effects of simultaneous use of cisplatin and acute radiation doses. Fitted radiation survival curves for each cell line without cisplatin (○) and combined with the indicated cisplatin dose (●). The results are the average of the actual data points and the bars represent one SD.

The poor outcome of patients with advanced or recurrent endometrial carcinoma shows that there is a demand for more effective treatment modalities. For this reason, chemotherapy has become a focus of interest. In vitro data obtained in the present study suggest that an additive cytotoxic effect can be achieved with the concomitant use of radiation and cisplatin in endometrial cancer. The potential enhancement of radiation damage in normal tissues must be studied before the therapeutic gain can be defined. Furthermore, other chemotherapeutic agents with a different mechanism of action than cisplatin should be tested.

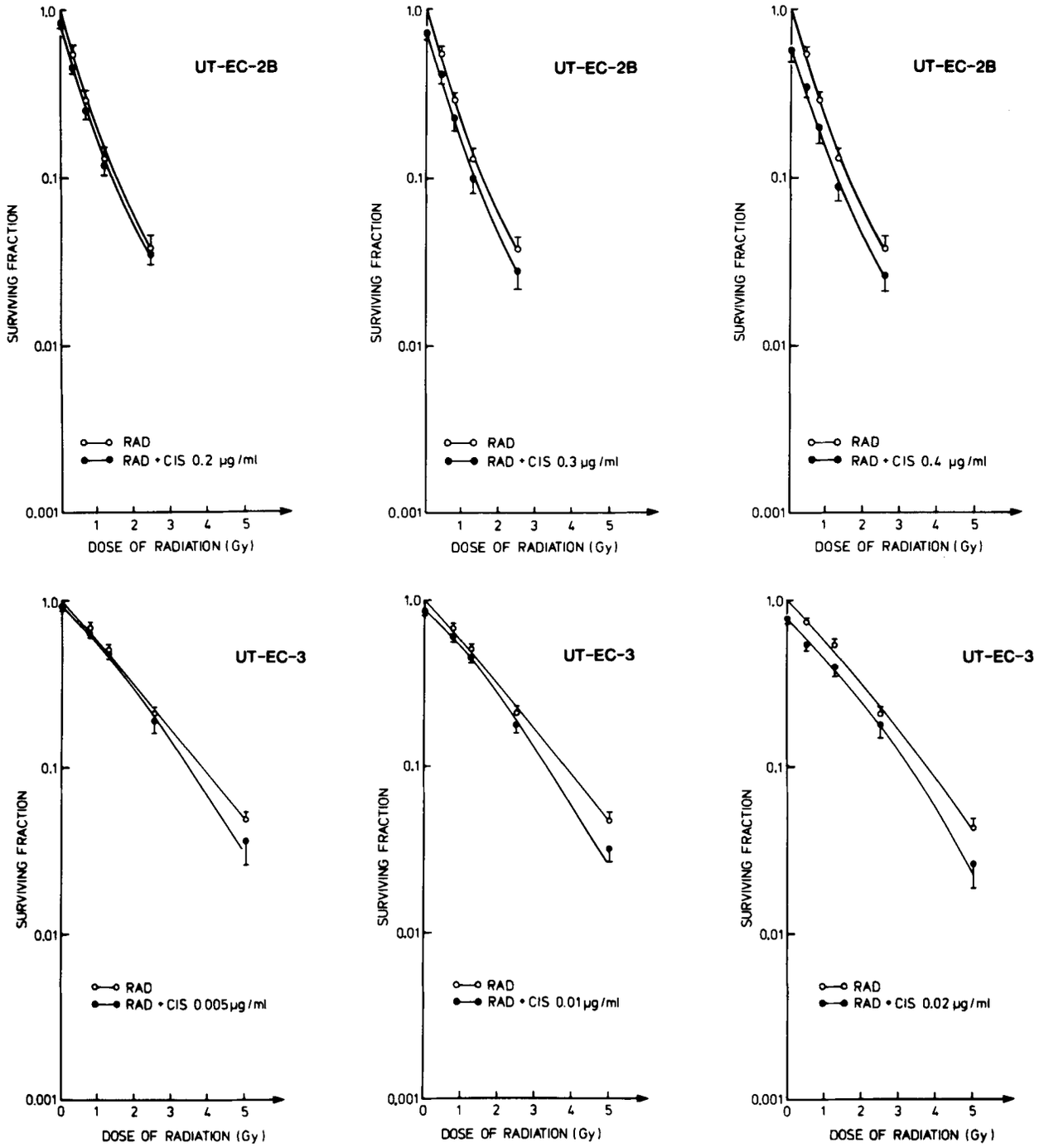

Fig. 1B. Effects of simultaneous use of cisplatin and acute radiation doses. Fitted radiation survival curves for each cell line without cisplatin (○) and combined with the indicated cisplatin dose (●). The results are the average of the actual data points and the bars represent one SD.

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