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Increase in *Cis*-Dichlorodiammineplatinum(II) Cytotoxicity upon Reversible Electropermeabilization of the Plasma Membrane in Cultured Human NHIK 3025 Cells*

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Abstract—*A series of brief electrical high-voltage discharges were given to cultured* NHIK 3025 *cells to render the plasma membrane transiently permeable to drugs. Using* [^{14}C]*sucrose as an inert marker which normally does not cross plasma membranes, increased permeability could be demonstrated for no longer than* 10 min *following electrical treatment, indicating that the permeabilization was entirely reversible. The reversibility of the treatment was further demonstrated by a lack of effect on cell growth and colony-forming ability. When cells were given electrical discharges immediately before or during exposure to* cis-*dichlorodiammineplatinum*(II)(cis-DDP) *the cytotoxic drug effect increased. By using electrical discharges during a* 2 hr *drug treatment period the cytotoxicity was enhanced to an extent corresponding to at least a* 3-*fold increase in drug uptake relative to unpermeabilized cells. This increase in drug uptake was confirmed by direct measurements of the amount of cell-associated* Pt *by atomic absorbtion spectroscopy. The results suggest that uptake across the plasma membrane may be the rate-limiting factor in the cytotoxic effect of* cis-*DDP. Furthermore, the methodology applied in the present study may prove useful in assessing the influence of membrane permeability on the effect of other cytotoxic drugs.*

INTRODUCTION

MANY chemotherapeutic drugs have been shown to exert their cytotoxic effect by inflicting damage upon vital macromolecules within the cell (i.e., DNA, tubulin, enzymes, etc.). Although any one such drug appears to inactivate different types of cells by the same molecular mechanisms, there is nevertheless considerable variation in the sensitivity of different cell types to the drug. The origin of such variations may be of a biological nature, reflecting differences in growth kinetics, repair mechanisms, the presence of protective compounds, tissue morphology, drug uptake through cell membranes, etc. Knowledge about the influence of these biological characteristics on drug toxicity may have great impact on both the development of new anticancer drugs, and the development of new treatment regimens for established drugs.

In the present study we have investigated the significance of the plasma membrane as a factor in limiting the cytotoxic effect of the chemotherapeutic drug *cis*-DDP. We have used an established electropermeabilization technique in an attempt to change the degree of permeability of the plasma membrane to this cytotoxic agent. It has been reported by others that the permeability of the plasma membrane to various chemical compounds can be increased, in a reversible manner, by exposing the cells to electrical discharges of high voltage and short duration and that such treatment results in little or no damage to intracellular organelles and cell function [1–5]. By using this method, substances which normally do not cross biological membranes, for example eosin and sucrose, can be introduced into the cytosol in large quantities [1, 5].

Our proposition was that if the cytotoxic effect of a drug like *cis*-DDP is in some way limited by the plasma membrane, the electrical discharge treatment applied in presence of the drug would facilitate the entry of drug into the cytosol thereby increasing the cytotoxic drug effect.

MATERIALS AND METHODS

Cells and cultivation

Cells of the established cell line NHIK 3025,

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derived from human uterine cervix carcinoma *in situ* [6, 7] were used. The cells were routinely grown as a monolayer, at 37° C in medium E2a [8] containing 30% serum [i.e., 20% human serum prepared in the laboratory and 10% horse serum (Gibco)]. In order to maintain cells in continuous exponential growth, the cell cultures were trypsinized (0.25% trypsin, Difco 1 : 250) and recultured three times a week [9]. Cells were routinely recultured on the day before use in experiments.

Electropermeabilization

The apparatus and method for electropermeabilization was, essentially, as previously described in detail [1]. Briefly, a 2 ml aliquot of cell suspension ($5–10 \times 10^5$ cells per ml of medium E2a) was placed in a square-bottomed (1×1 cm) Perspex chamber, two of the opposing inner walls of which were stainless steel electrodes. The electrodes were connected to a 1.2 μF capacitor which was charged by a 2000 V power supply. By using a manual switch the charged capacitor could be discharged through the circuit containing the electrode chamber. Under these conditions the time constant of the exponential decay was found to be about 45 μsec. Consecutive discharges were given at intervals of about 3 sec. The above experimental procedures were carried out in an incubator room maintained at 37° C.

Estimation of [^{14}C]sucrose uptake

15 μCi[^{14}C]sucrose was added to 1 ml suspensions of electropermeabilized or non-electropermeabilized NHIK 3025 cells at 37° C. After 20 min, 300 μl samples of cell suspension were washed three times in 4 ml volumes of ice-cold phosphate-buffered saline (PBS), pelleting by centrifugation between washes. Washed cell pellets were dissolved in 500 μl 0.4% (w/v) deoxycholate in 0.1 M NaOH, thereafter mixed with 3 ml scintillation fluid plus 150 μl 1 N HCL, and radioactivity was measured as DPM (disintegration per minute) in a liquid scintillation counter (Beckman LS 1800, U.S.A.).

Atomic absorbtion spectroscopy

After treatment with drugs and/or permeabilization cell suspensions were washed once in E2a and to each sample was added 200 μl 8N HNO_3 per 10^6 cells. Thereafter the samples were sonified for about 15 sec with a Branson Cell Disruptor B15. Analysis of cellular-bound platinum was performed using a Varian SpectrAA-30 atomic absorbtion spectrometer fitted with a GTA-96 graphite tube atomizer. Instrument control and data acquisition was by Varian DS-15 Data Station using Varian Atomic Absorbtion Software. Fifty μl aliquots were placed in a graphite tube and the atomic absorbtion signal measured with a platinum-lamp at 256.9 nm by using a suitable temperature-program. Automatic background correction with a modulated deuterium lamp was utilized. The amount of platinum was calculated from a calibration curve run immediately before samples. Each point was represented by three parallels from which the mean value and standard error (S.E.) was calculated.

Increase in cell number

To monitor cell proliferation capability after electrical treatment the increase in cell number was registered: cells were loosened from stock cultures by trypsinization, exposed to electrical discharges and then seeded into Falcon plastic flasks (25 cm^2 growth area). At different times afterwards, the mean number of cells per microcolony was calculated by differential counting of about 100 microcolonies utilizing an inverted microscope.

Cell survival measurements

Cell survival was estimated from colony forming ability: after trypsinization and centrifugation, the NHIK 3025 cells were resuspended in medium E2a ($5–10 \times 10^5$ cells/ml). The cells were kept in suspension while they were treated with *cis*-DDP (2 hr) and/or exposed to electrical discharges. Treatment with *cis*-DDP was initiated by adding a small volume of a concentrated solution of drug, in glycozole [10] to the cell suspension. Following exposure to the drug, the cells were transferred to fresh medium and a cell number adjusted to give 150 colonies per dish was seeded into 6 cm Falcon plastic dishes with 5 ml fresh medium. The dishes were incubated in an atmosphere of 5% CO_2 at 37° C for a total of 10–14 days with a medium change on day 6. Cells in the dishes were subsequently fixed in 96% ethanol and stained with a saturated solution of methylene blue. Only colonies containing more than 40 cells were considered as originating from a cell which had survived the experimental manipulations. Each observation was represented by five dishes from which the mean value and standard error (S.E.) were calculated.

Drugs

Cis-DDP (*cis*-dichlorodiammineplatinum) was purchased from Farmitalia Carlo Erba, Spain, and [^{14}C]sucrose was purchased from the Radiochemical Centre, Amersham, Bucks, U.K.

RESULTS

In Fig. 1, the broken line represents the amount of cell-associated [^{14}C]sucrose in control cells after a 20 min incubation at 37° C; data are given as DPM per cell sample. The circles represent the

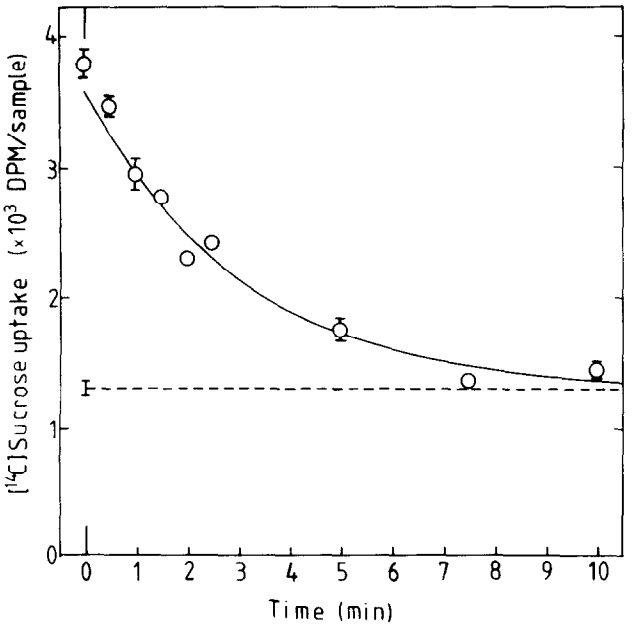

Fig. 1. Uptake of [^{14}C]*sucrose by NHIK* 3025 *cells. The cell received five consecutive electrical discharges and at different times thereafter as indicated on the asbscissa* [^{14}C]*sucrose was added. The isotope was present for* 20 min *before the activity taken up by the cells was measured. The stippled line [with standard error (S.E.)] shows the uptake by cells that did not receive electrical discharges. Each experimental point is represented by three parallel samples from which the mean value was calculated. S.E. is indicated when exceeding the symbols. The curve was fitted by the method of least squares for an exponential decrease in drug uptake and* $t_{1/2}$ *was calculated to* 2.1 min (*corr.coef.* = 0.94).

corresponding radioactivity in samples of cells exposed to five consecutive electrical discharges (pulses) at time 0, then incubated for a 20 min period with [^{14}C]sucrose starting at the time points indicated on the abscissa. While the data indicate that there is a significant uptake of [^{14}C]sucrose into control cells, the amount of radioactivity which enters electropermeabilized cells is 3-fold greater when the [^{14}C]sucrose is added immediately after the electrical treatment. However, the amount of [^{14}C]sucrose taken up into cells decreases quickly as the time between electrical discharges and addition of [^{14}C]sucrose is increased, and is down to the control level after 5–10 min.

The data of Fig. 1 indicate that the membrane damage induced by the electrical discharge treatment is reversible. It was therefore of interest to investigate the effect of electrical discharges on cell survival and cell growth kinetics. In Fig. 2, cell survival is shown as a function of the number of consecutive discharges to which the cells were exposed. The survival curve is characterized by an initial shoulder up to about 10 discharges, where no significant effect of the treatment can be seen, followed by an exponential decrease in cell survival.

Cell growth was measured as the increase with time in the mean cell number per microcolony (Fig. 3). For cells exposed to up to 15 discharges, the data show no significant reduction in the mean doubling time as compared to untreated cels. Thus, even when cells are given 15 consecutive discharges, after which only about 50% are able to form colonies (Fig. 2), cell cycle progression is not delayed in the surviving cells.

From Fig. 1 it can be seen that cellular uptake of [^{14}C]sucrose is greatly enhanced during the first minutes after the electrical discharges. The probable reason for this is that the electrical treatment causes electro-mechanical instabilities in the cell membrane resulting in a transient increase in its permeability to small molecules [2, 3, 11]. With this in mind, we studied the effect of cell survival of *cis*-DDP (5 μM, 2 hr exposure) added at various times after the cells were exposed to five electrical discharges. Figure 4 shows cell survival as a function of time elapsing between electrical discharges and start of a 2 hr drug treatment; the broken line represents the fraction of cells surviving a 2 hr treatment with 5 μM *cis*-DDP in the absence of any electrical discharges. The cytotoxicity of *cis*-DDP was considerably greater when the drug was added immediately after the cells received the electrical discharges. However, when *cis*-DDP was added at subsequent time points its cytotoxicity gradually diminished to the level seen in cells which had not received electrical discharges.

The results depicted in Fig. 4 may be explained satisfactorily by supposing that the uptake of *cis*-DDP is increased during the first minutes after the electrical discharges, i.e., that the electrical treatment increases the cell membrane's permeability for *cis*-DDP as it does for [^{14}C]sucrose (Fig. 1). However, the membrane damage must

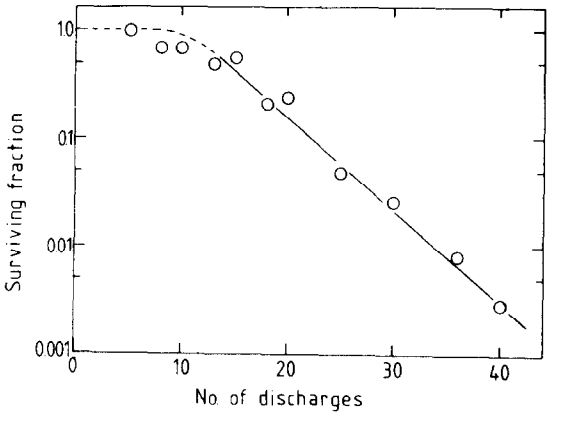

Fig. 2. Survival of NHIK 3025 *cells as a function of the number of consecutive discharges. The straight line was fitted by the method of least squares for cells that received more than* 10 *discharges (corr.coef.* = 0.99, D_0 = 5 *discharges, Dq* = 11 *discharges) and the stippled line was fitted by hand. Standard errors (S.E.) did not exceed the symbols.*

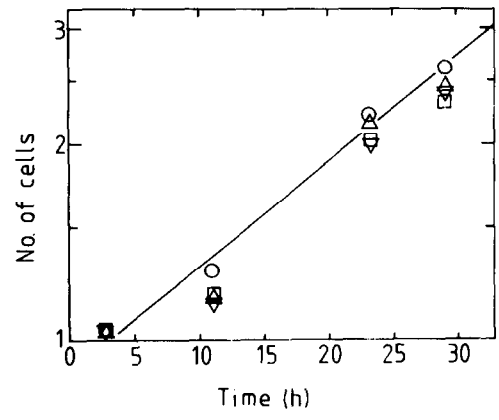

Fig. 3. The mean number of NHIK 3025 cells per microcolony as a function of the time after seeding of single cells. Before seeding the cells received either no discharges (control) (○), 5 discharges (△), 10 discharges (▽) or 15 discharges (□). The straight line was fitted by the method of least squares (corr.coef. = 0.99) for cells that did not receive electrical discharges (control) and it shows an exponential increase in cell number with a mean doubling time of 18.9 hr.

be restituted rapidly, since the fraction of cells surviving drug treatment increases if some minutes elapse between electropermeabilization and start of *cis*-DDP treatment.

While Fig. 4 shows that cell survival was lowered from 22% to 1% (values at time = 0 in Fig. 4) for cells exposed to just one sequence of five

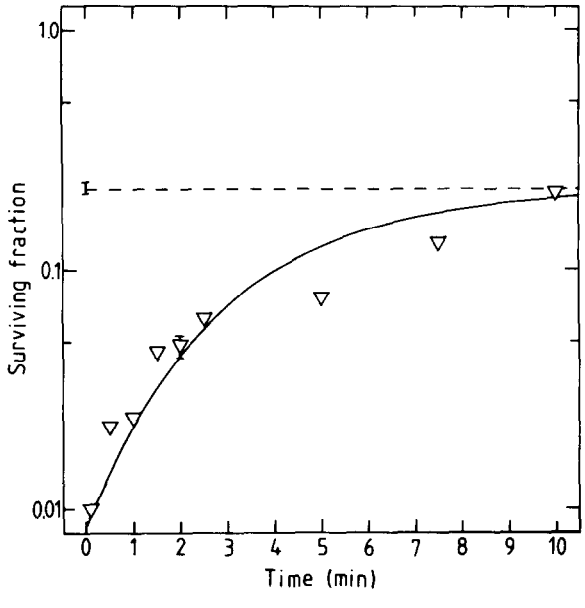

Fig. 4. Survival of NHIK 3025 cells after treatment with 5 μM cis-DDP for 2 hr. The cells were given five electrical discharges and cis-DDP was added at different times thereafter as indicated on the abscissa. The stippled line [with standard error (S.E.)] shows the survival for cells that did not receive electrical discharges. The curve was fitted by the method of least squares for an exponential decrease in ln S(t)-ln S_0 *where* S_0 *is the surviving fraction for cells treated with* cis-*DDP that did not receive electrical discharges and* S(t) *the surviving fraction for cells that received* cis-*DDP at time* t *after the electrical discharges were given.* $t_{1/2}$ *was calculated to* 2.0 min *(corr.coef. = 0.93). Standard errors (S.E.) are indicated when exceeding the symbols.*

consecutive electrical discharges immediately prior to the addition of *cis*-DDP (5 μM, 2 hr exposure), the data of Fig. 5 show that the effect of *cis*-DDP is even further increased if the cells are given repeated electrical discharges during drug treatment. Furthermore, to support our assumption that the cytotoxicity was a result of an increased drug uptake we also measured the amount of cell associated Pt (Fig. 5, lower panel). In these experiments samples of cells were exposed to a standard electrical discharge treatment (consisting of five consecutive discharges, each single discharge given at intervals of about 3 sec) either once, twice or up to 6 times in the course of the 2 hr period during which *cis*-DDP was present in the cell culture. The interval between the standard discharge treatments was $120/n$ min, where n is the number of standard discharge treatments applied. As shown in Fig. 5 (upper panel), up to six series of electrical pulses had relatively little effect on the survival of cells that did not receive *cis*-DDP. However, for cells treated with 3 or 4 μM *cis*-DDP, cell survival fell progressively as cells were exposed to an increasing number of standard discharge treatments. Series of electrical pulses in excess of the first three or four did not further significantly increase the sensitivity of the cells to the applied doses of *cis*-DDP.

From the lower panel of Fig. 5 the amount of cell associated Pt as measured by atomic absorbtion spectroscopy was found to increase with increasing numbers of standard discharge treatments up to the first three or four treatments (Fig. 5, lower panel). Thereafter the amount of cell associated Pt was found to decline. This may be explained by the effect seen from the upper panel in Fig. 5, that there is a significant effect on cell survival by the electrical treatment alone (after six treatments about 50% of the cells are killed). Thus the decrease in cell-associated Pt observed after more than four electrical discharge treatments is probably a result of irrepairable membrane disruption caused by too many electrical discharges.

In order to investigate whether the electrical treatment in itself modified the cytotoxicity of *cis*-DDP, an experiment was conducted in which a solution of *cis*-DDP was exposed to five consecutive electrical discharges immediately before it was added to the cells. In so far as there was no detectable change in the colony forming ability of cells exposed to "normal" or "pulsed" *cis*-DDP, this treatment did not alter the cytotoxicity of *cis*-DDP *per se*. Thus, it is likely that *cis*-DDP remained unchanged by the electrical discharges applied in the above experiments in which up to six series of pulses were utilized.

In Fig. 6 a comparison is made between the effect of exposing NHIK 3025 cells to various concentrations of *cis*-DDP for 2 hr, either in the

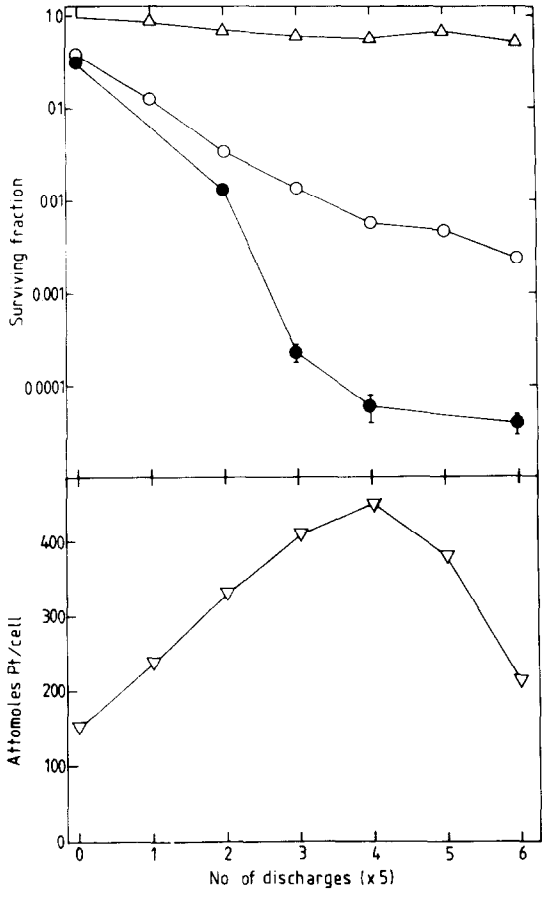

Fig. 5. Survival (upper panel) and the amount of cell associated Pt (lower panel) for NHIK 3025 cells that received electrical discharges during treatment with cis-*DDP for* 2 hr. *Survival of cells were measured after treatment without control* (△) *and with* 3 (○) *or* 4 (●) μM cis-*DDP. Uptake of Pt* (▽) *was measured after treatment with* 20 μM cis-*DDP. Five consecutive electrical discharges were given* n *times as indicated on the abscissa* (n = 1–6) *each* 120/n min *from the start of the* cis-*DDP treatment. Standard errors (S.E.)are indicated when exceeding the symbols.*

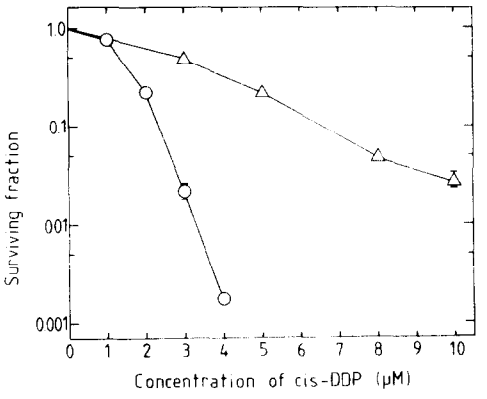

Fig. 6. Survival of NHIK 3025 *cells after treatment with different concentration of* cis-*DDP for* 2 hr. *The cells received no discharges* (△) *or five consecutive discharges* 3 *times each* 40 min *from the start of the* cis-*DDP treatment* (○). *Standard errors (S.E.) are indicated when exceeding the symbols.*

absence of electrical treatment (triangles) or when standard discharge treatments were applied at 40-min intervals [i.e., at time points 0, 40 and 80 min (circles)]. Clearly, throughout the range of *cis*-DDP concentrations investigated, cells which received electrical discharges were much more sensitive to *cis*-DDP than were those which did not. Although we cannot exclude the possibility that the small difference in shape between the two dose-effect curves shown may be of significance one may as a good approximation state that the difference between the two curves represents a 3-fold dose-enhancing effect.

DISCUSSION

Effect of electrical discharges on cell growth, cell survival and plasma membrane permeability to sucrose

The apparent uptake of [^{14}C]sucrose by cells which were not electropermeabilized (Fig. 1) may be related to the endocytotic activity of NHIK 3025 cells and to the accumulation of sucrose at the cell surface region due to trapping and adsorption [1]. By exposing NHIK 3025 cells to electrical discharges of high voltage and short duration they could be rendered permeable to [^{14}C]sucrose (Fig. 1). However, upon subsequent incubation at 37° C, the cell's permeability to sucrose was seen to decline in an exponential manner with $t_{\frac{1}{2}}$ of about 2.1 min. This exponential decay in membrane permeability is similar to that which has been observed in rat hepatocytes [1] and in mouse lymphocytes [5] permeabilized by the same procedure. That such widely different cell types respond to this electrical treatment so similarly, implies that a common structural alteration in the plasma membrane must arise, which is responsible for the permeability changes seen [2]. A so-called "dielectric breakdown" of the membrane occurs above a certain critical voltage, and results in regional changes in the lipid bi-layer of the plasma membrane leading to enhanced membrane permeability [2, 3, 11].

Various studies on the changes in the plasma membrane brought about by electropermeabilization indicate that it became transiently permeable to molecules of low MW [1, 2, 5]. Clearly then, a loss of ions and compounds of low MW from the cell might have an adverse effect upon normal cell function. However, electropermeabilization did not seem to affect NHIK 3025 cells adversely, provided that the number of electrical pulses applied to the cells was limited to 10 or less. Under these conditions, normal membrane permeability was restored rapidly—within 5–10 min at 37° C—and no effect on growth rate and cell survival was detectable (Figs. 2 and 3). It is therefore most probable that any effects of up to 10 electrical discharges on normal cellular function are slight, and that they are fully reversed within

a short period following the restoration of normal permeability of the plasma membrane. Under conditions where cells were exposed to more than about 10 discharges, cell survival decreased in an exponential manner with increasing number of discharges (Fig. 2). The most likely explanation for this is that each pulse produces a certain amount of membrane damage and that the effect of consecutive pulses is additive. Consequently, cell death will ensue when membrane damage passes a certain critical level, either because the membrane becomes mechanically unstable and does not repair [1] or because the time taken to effect repair of the plasma membrane is so long that osmotic processes produce cell lysis first [4]. When cells were subjected to separate series of 5 consecutive discharges at intervals of at least 20 min the effect on cell survival was much less pronounced (Fig. 5). The probable reason for this is that the damage to the plasma membrane inflicted by each series of discharges was fully repaired before the next series was given.

Effect of electrical discharges on plasma membrane permeability to cis-*DDP*

The cytotoxicity of *cis*-DDP was clearly increased if the drug was present immediately after (Fig. 4) or during treatment with electrical discharges (Figs. 5, upper panel and 6). The fact that the amount of cell associated Pt as recorded by atomic absorbtion spectroscopy was increased after treatment with electrical discharges (Fig. 5, lower panel) strongly supports the conclusion that the increased cytotoxicity is due to an increased uptake of *cis*-DDP through plasma membrane. Other explanations such as for example that the cellular metabolism may be altered due to the electrical discharges seem unlikely since the effect of *cis*-DDP was found to be increased even after discharge treatments which by themselves did not affect either cell survival or cell cycle progression (Figs. 2 and 3).

An interesting question is, however, whether or not the restitution of membrane damage affects the uptake of [^{14}C] sucrose and *cis*-DDP similarly. From Fig. 4 cell survival was found to increase with time elapsed between treatment with electrical discharges and addition of drug. The survival curve in Fig. 6 representing cells treated with *cis*-DDP without electrical discharges (upper curve), is well fitted by a straight line in the drug dose range from 3 to 10 μM, the correlation coefficient being 0.994. Thus, in this dose range the curve is exponential. Furthermore, atomic absorbtion measurements have shown that the amount of cell associated Pt is proportional to the extracellular concentration in this concentration range (data not shown). It therefore seems reasonable to assume that the survival (S) is proportional to an exponential function:

$$S \propto e^{-[C]}$$
$$-\ln S \propto -[C]$$

where $[C]$ is the intracellular concentration of *cis*-DDP. Thus, ln$S$ may be a relative measure of the amount of intracellular *cis*-DDP, and if our assumption is correct, Fig. 4 describes the decrease in the rate at which *cis*-DDP is taken up into cells with increasing time from electropermeabilization to addition of drug. The data of both Fig. 1 and Fig. 4 are, within the limits of experimental error, well fitted by exponential functions with similar half times. Thus, the cell membrane is restituted in a manner which probably reduces its permeability for *cis*-DDP and for [^{14}C]sucrose at the same rate.

The effect of *cis*-DDP was enhanced optimally when cells were exposed to a series of five consecutive electrical discharges four or more times, at regular intervals, during the 2-hr incubation with the drug present (Fig. 5, upper panel). This indicates that an equilibrium is reached between the intracellular and extracellular drug compartments and that no further increase in the intracellular amount of drug is possible for this concentration of drug in the medium. Using atomic absorbtion measurement the amount of Pt/cell was calculated (Fig. 5). Because some of the cells were disrupted by the electrical discharge treatment, (see the upper panel in Fig. 5), our measurement may represent an underestimation. This is obviously the case for cells treated with as many as five and six standard discharge treatments (see the lower panel in Fig. 5) and a similar effect was reported for the uptake of [^{14}C]sucrose in rat hepatocytes [1]. For cells treated with three standard discharge treatments the amount of cell associated Pt is 2.7 times higher than for untreated cells. A comparison between the *cis*-DDP dose-response curves for unpermeabilized and permeabilized cells that received three standard discharge treatments showed a dose-modifying factor of about 3 (Fig. 6). It therefore seems probable that the uptake of *cis*-DDP through the plasma membrane of NHIK 3025 cells is increased 3-fold by this treatment.

The transport of cis-*DDP into mammalian cells*

The ultimate cellular target for *cis*-DDP is believed to be DNA [12, 13]. Our results suggest that the factor which limits the access of *cis*-DDP to its target is the functional integrity of the plasma membrane. While the exact mechanisms responsible for the passage of *cis*-DDP from the extracellular environment to its ultimate site of action within the nucleus are unknown, our results suggest that, under normal conditions, only a limited

amount of *cis*-DDP is able to cross the plasma membrane and gain access to the cytosol. We have previously found that the cytotoxicity of *cis*-DDP is substantially reduced at temperatures below 37° C, an observation which accords with that of others [14, 15], and that its cytotoxicity is also diminished in our cells under hypoxic conditions (unpublished results). Although the possible explanations to the reduced cytotoxicity of *cis*-DDP at hypothermic temperatures is numerous [15], these results suggest that *cis*-DDP may be taken up into the cell by a transport mechanism which is both temperature- and energy-dependent, i.e. by some form of active transport. This is in accordance with the suggestion of Byfield and Calabro-Jones [14, 16] that *cis*-DDP is taken up by amino acid carrier proteins. Scanlon *et al.* [17, 18] found that the uptake of methionine and aminoisobutyric acid in L1210 cells was reduced by *cis*-DDP. They suggested that a possible explanation could be that *cis*-DDP was bound to specific membrane carriers.

Safirstein *et al.* [19] have shown that the uptake of ethylene-diamminechloroplatinum (an analogue of *cis*-DDP) into rat kidneys is temperature- and energy-dependent, a finding which also suggests the existence of an active transport mechanism. When the uptake of *cis*-DDP into kidneys are inhibited by certain drugs [19, 20] the reason could be that the active transport is in some way influenced by these drugs. Similarly our observation [21] that benzaldehyde, an anticancer drug which binds to membrane proteins [22, 23], reduces the cytotoxicity of *cis*-DDP could mean that benzaldehyde influences the active transport.

In some earlier reports, it was proposed that *cis*-DDP and another platinum-analogue *cis*-dichloro(dipyridine)platinum may enter the cells by passive diffusion [24, 25]. This proposition was partly based on the observation that, in haemopoietic precursor cells, the cytotoxicity of *cis*-DDP was largely temperature-independent, while the cytotoxicity of a nitrogen mustard (HN2) was strictly temperature-dependent [25]. However, other workers dispute that the cytotoxicity of *cis*-DDP is temperature-independent [15]. Such conflicting results could possibly suggest that *cis*-DDP is transported by different mechanisms according to cell type. The results of Ogawa *et al.* [25], which imply separate modes of transport for *cis*-DDP and HN2, have been strengthened by the results of Dornish *et al.* [21] who also find it likely that *cis*-DDP and HN2 are taken up into the cells by entirely different mechanisms. In the case of cells lacking an active transport mechanism that are functional for *cis*-DDP its cytotoxic effect may perhaps be related to simple passive diffusion [14].

While the fact that some cell types are less sensitive to *cis*-DDP than others may be related to differences in their ability to repair drug-induced DNA damage [26, 27], our results suggest that the degree to which the drug passes through the plasma membrane could be of equal importance. It can be imagined that in an *in vivo* situation tumour cells which are particularly sensitive to *cis*-DDP could be those having an effective transport of the drug.

The results of our experiment suggest that the electropermeabilization technique described above may be useful in investigating the role of the plasma membrane in determining the cytotoxicity of certain drugs.

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