# Photolytic degradation of adriamycin

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Adriamycin (doxorubicin hydrochloride, NSC 123127) (ADR), an antibiotic widely used in cancer chemotherapy (Tan et al 1973; Blum 1975), consists of an anthracycline moiety and an amino sugar, daunosamine, linked together through a glycosidic bond. Like related anthracycline antibiotics, ADR is believed to be photosensitive. However, no photolytic studies on this group of compounds have been published, and warning of their possible photodegradation has been reported only in the clinical brochure offered by the manufacturer. This report concerns the kinetics of degradation of ADR solutions when exposed to fluorescent light.

ADR bulk powder was obtained from the Division of Cancer Treatment, NCI, NIH, and was chromatographically purified (>95%) before use (Tavoloni & Guarino 1980). ADR solutions in various solvents were prepared in Pyrex-glass scintillation vials closed tightly with plastic caps. For each solvent, 6 ADR concentrations ranging from 10 to 500 $\mu$g ml$^{-1}$ were prepared. All samples were prepared in triplicate and protected from light with aluminium foil before each study. The ADR concentration of each solution was determined fluorometrically (Bachur et al 1970), and ADR photodegradation, resulting from breakdown of the naphthacenequinone nucleus, by measuring the loss of fluorescence over time. In all instances, fluorescence values were determined in triplicate and the mean for each determination recorded.

The photolysis of ADR was studied under three different experimental conditions. For each set of triplicate vials, one was kept in absolute darkness, one exposed to room light and one irradiated with intensive light. Vials of ADR solutions kept in the dark remained covered with aluminium foil and stored in a dark room. In room light studies, vials were kept upright on a table in the laboratory and light was provided by 12 40-watt ceiling fluorescent-tubes, mounted approximately 2 m above the vials. In intensive light studies, samples were exposed to room light as above, and simultaneously irradiated with 2 additional desk lamps, each equipped with two 15-watt tubes, positioned on either side of the vials 10–20 cm from the samples.

Samples were assayed for fluorescent activity at different intervals for 144 h. Where photodegradation of ADR was very rapid, fluorescence was determined as often as every 2 min, whereas in other more stable solutions fluorometric determinations were made at 3, 6, 12 or 24 h intervals.

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All fluorescence activities are expressed as percentages of the initial value obtained at time 0, and represent a mean of 3 values obtained from three separate studies performed within a 30 day period. Data were analysed for first order kinetics by fitting to the equation $Y = Y_0e^{-kt}$, where $Y_0$ is the fluorescence at zero time, k the exponential rate constant and t is time. The half-life value was calculated as: $T\frac{1}{2} = 0{\cdot}693/k$.

At a concentration of 50 $\mu$g ml$^{-1}$ in distilled water, ADR lost all of its fluorescence activity during 30 h of exposure to intensive light (Fig. 1). In room light, the drug solution was significantly more stable, although 67% of the initial fluorescence was lost at the end of 144 h. When the ADR solution was kept in the dark, no photodegradation was observed.

While the stability of the ADR solution *in the dark* is independent of the concentration in the range 10–500 $\mu$g ml$^{-1}$, the rate of degradation observed when the drug is exposed to light is inversely proportional to the concentration used. In samples exposed to room light, rapid photolysis occurred when ADR was dissolved in distilled water at concentrations ranging from 10–50 $\mu$g ml$^{-1}$ (Fig. 2). Beyond 100 $\mu$g ml$^{-1}$, little or no photodegradation was observed. When samples of ADR were

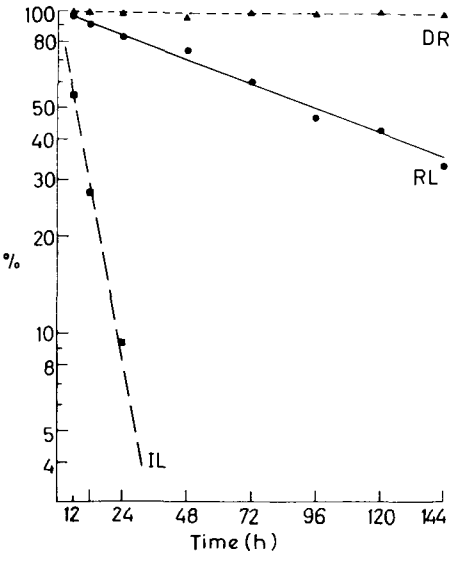


FIG. 1. Loss of fluorescent activity of ADR solutions exposed to room light (RL), intensive light (IL) or kept in the dark (DR). ADR was dissolved in distilled water at 50 $\mu$g ml$^{-1}$. Values are percentages of the fluorescent activity at time 0. Lines represent the best computer fit of the experimental data to the equation $Y = Y_0e^{-kt}$ (see methods).

Table 1. Adriamycin half-lives (a).

| Exposure | Solvent | Concentration ($\mu$g ml$^{-1}$) 10 | 25 | 50 | 100 | 250 | 500 |
|---|---|---|---|---|---|---|---|
| Room light | Water | 21·72 | 36·47 | 94·15 | (69) | (78) | (96) |
| | Saline | 23·65 | 60·79 | 119·90 | (74) | (84) | (99) |
| | Ringer's(b) | 8·02 | 14·08 | 20·14 | 65·75 | 137·14 | (56) |
| | Ethanol | (56) | (86) | (100) | (100) | (100) | (100) |
| | Bile | (100) | (100) | (100) | (100) | (100) | (100) |
| Intensive light | Water | 0·89 | 1·29 | 6·72 | 33·48 | (55) | (76) |
| | Saline | 0·82 | 1·54 | 8·11 | 37·86 | (57) | (85) |
| | Ringer's(b) | 0·28 | 0·53 | 2·49 | 7·25 | 47·1 | 65·6 |
| | Ethanol | 19·04 | 85·58 | (63) | (89) | (100) | (100) |
| | Bile | (60) | (100) | (100) | (100) | (100) | (100) |

(a) Values are expressed in hours and represent the mean of three determinations. Values in parentheses are percentages of the initial fluorescent activity detected at the end of 144 h exposure (kinetic analysis was not done on these data because one experimental half-life could not be determined during the experimental period).
(b) Ringer-Krebs bicarbonate.

irradiated by intensive light, however, photodecomposition was more pronounced at all concentrations tested (Table 1). Thus, at 10 $\mu$g ml$^{-1}$ in distilled water, a half-life as short as 0·89 h was obtained with intensive light, compared with 21·7 h when the same sample was exposed to room light. Under intensive light, photodegradation occurred even at a concentration of 500 $\mu$g ml$^{-1}$, and approximately 25% of the initial fluorescence was lost at the end of the 144 h exposure period (Table 1).

Photodegradation of ADR is also dependent upon the nature of the solvent used (Fig. 3 Table 1). Thus, while ADR is most strongly protected from photolysis when dissolved in fresh rat bile, maximal decomposition occurred when the drug is dissolved in Ringer-Krebs bicarbonate. With respect to their protective effect, the solvents tested demonstrated the following order: bile $\gg$ ethanol $>$0·9% NaCl (saline) $>$ distilled water $>$ Ringer-Krebs bicarbonate. Such a sequence was found to be independent of the concentration of ADR and of

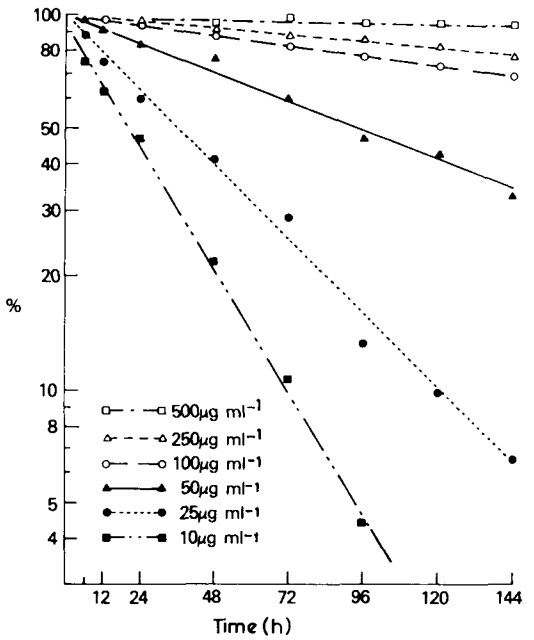


FIG. 2. Loss of fluorescent activity of ADR solutions exposed to room light. All concentrations of ADR were prepared in distilled water. See Methods and Legend to Fig. 1 for details.

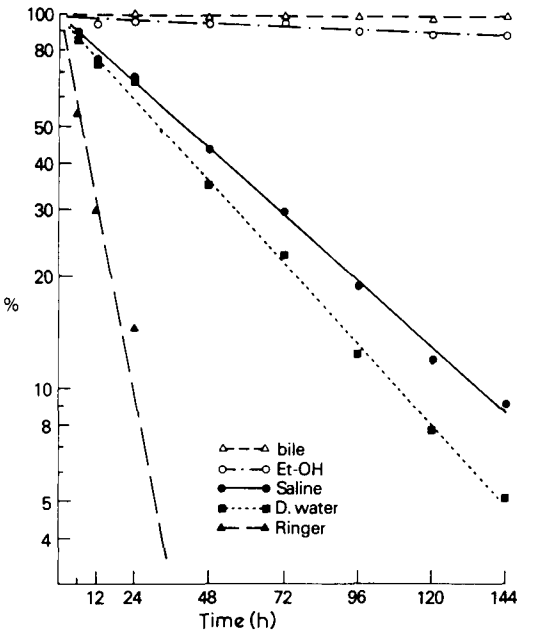


FIG. 3. Loss of fluorescent activity of ADR solutions exposed to intensive light. In all instances, ADR was dissolved at 100 $\mu$g ml$^{-1}$. See Methods and Legend to Fig. 1 for details.

the intensity of the light used. Table 1 summarizes the half-lives of the ADR solutions when exposed to room light or intensive light. Irrespective of the solvent used, no photodecomposition was observed in samples of ADR kept in the dark.

The objectives of these studies were three-fold: to determine whether ADR is photo-degradable, to study the kinetics of ADR photodegradation and to examine possible conditions which may facilitate or prevent its decomposition. The data indicate that ADR is, in fact, extremely photosensitive. Exposure of drug solutions to light resulted in loss of fluorescent activity in a fashion proportional directly to the intensity of the light irradiated and inversely to the concentration of the drug. In all instances, photodegradation of ADR within the 144 h exposure period followed first order kinetics. Inasmuch as the experimental conditions of the present study are similar to those in which the drug is handled for clinical or laboratory use, the present findings are of practical importance and indicate that decomposition of ADR may be significant if the drug in solution is exposed to light for a sufficient period of time.

Concerning the possibility of preventing the decomposition of ADR, important observations emerge from the present studies. Keeping the ADR solution in absolute darkness effectively protected the drug from degradation, irrespective of the concentration and solvents used. This indicates that decomposition of ADR is virtually entirely photolytic, and that, providing exposure to light is avoided, the drug can be stored safely in solution for at least a week at concentrations of 10 $\mu$g ml$^{-1}$ or greater. Moreover, photodegradation of ADR is dependent upon the nature of the solvent used. Dissolving ADR in absolute ethanol or rat bile resulted in partial or nearly complete protection respectively, even when the drug was exposed to intensive light. Solutions of ADR in Ringer-Krebs bicarbonate were by far more sensitive to photodegradation. At present, no plausible explanation can be offered for these differences. Although it is possible that, the yellow-green colour may be responsible for the protective effect of bile, chemical or physicochemical interactions between ADR and the solvent or components of the solvent may occur and modify the photosensitive characteristics of the antibiotic molecule. Irrespective of the mechanism by which the solvent influences the photodegradation of ADR, however, the protective effect of the bile is of particular interest in view of the extensive biliary excretion of the drug (Bachur et al 1974; Riggs et al 1977; Israel et al 1978). Bile was chosen as a solvent in the present studies in part because we recently found (Tavoloni & Guarino 1980) that the biliary elimination of total ADR equivalents in anaesthetized rats is significantly higher than that previously reported in conscious rats (Yesair et al 1972; Israel et al 1978). Since we had speculated that photodegradation might have caused the lower biliary concentrations of the drug found previously, it was essential to clarify this possibility. By showing that the bile protects ADR from photolysis, the present findings invalidate our previous speculation and indicate that other factors must underlie the controversial results.

In conclusion, the present studies indicate that ADR in solution is photodegradable and that, at concentrations lower than 500 $\mu$g ml$^{-1}$, appreciable loss of biochemical activity occurs if exposure to light is not prevented. Mainly because of the higher concentrations (2 mg ml$^{-1}$) which are prepared when ADR is given to cancer patients, the data suggest that no special precautions are necessary to protect freshly prepared ADR solutions from light during intravenous administration. However, because photo-degradable concentrations may be obtained in vitro or in tissues and body fluids in experimental situations, the importance of these results to laboratory studies on ADR must be kept in mind.

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