Defective Repair of Tryptophan Pyrolysate (Trp P-1 and Trp P-2) and Aflatoxin B1 Damage in Xeroderma Pigmentosum Cells

TOYO OKUI** and YOSHISADA FUJIWARA***

Hokkaido Institute of Public Health,**
Kita-19, Kita-ku, Sapporo 060, and
Department of Radiation Biophysics,***
Kusunoki-cho 7-5-1, Chuo-ku, Kobe 650, Japan

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Optimally activated aflatoxin B1 (AFB1), 3-amino-1,4-dimethyl- and 3-amino-1-methyl-5H-pyrido[4,3-b]indoles (Trp P-1 and Trp P-2) exerted more lethal effects on xeroderma pigmentosum (XP) complementation group G cells than on normal human cells. XP group G cells exhibited deficiency in unscheduled DNA synthesis and in accumulation of arabinofuranosyl cytosine/hydroxyurea-induced single strand breaks after treatment with the three agents. Thus, Trp P-1 or Trp P-2 damage can be repaired mainly by nucleotide excision repair, as found with AFB1 damage. Activated AFB1 induced more sister chromatid exchanges (SCEs) in XP group G cells than in normal cells, while such a differential response to SCE was not observed with activated Trp P-1 and Trp P-2.

INTRODUCTION

Mycotoxin aflatoxin B1 (AFB#) and tryptophan pyrolysis principles Trp P-1 and Trp P-2 are not only mutagenic to bacterial1-4) and mammalian5-7) cells, when metabolically activated with liver microsomal enzymes, but also oncogenic to mammals.7-9) Basically, activated AFB1 metabolite forms covalent DNA-adducts, which can be repaired by the uvrA and recA systems1) and induces...
an error-prone repair\textsuperscript{10} in \textit{Escherichia coli}. It also induces repair synthesis in normal human cells, but not in XP complementation group A cells,\textsuperscript{11} which are defective in nucleotide excision repair of UV damage.\textsuperscript{12,13} On the other hand, \textit{N}-hydroxy-Trp P-2 among multiple metabolites by microsomal P-450 is involved in the covalent binding to DNA.\textsuperscript{14,15} However, little is known about the repair of DNA lesions induced by Trp P-1 and Trp P-2 in human cells. In this short report, we describe the lethal and SCE-inducing effects of Trp P-1 and Trp P-2, and the repair of their lesions in normal and XP group G cells, in comparison to relatively well-known AFB1 damage.

MATERIALS AND METHODS

The diploid fibroblast strains used were NHSF46 (normal human)\textsuperscript{16} and excision-defective XP2BI complementation group G\textsuperscript{12,13} (kindly supplied by Dr. D. G. Harnden, University of Birmingham, UK). Cells were grown in Eagle's minimal essential medium with 10–15\% fetal calf serum (Flow Lab., Maryland), as described previously.\textsuperscript{16}

AFB1 (Sigma Chemical Co.), Trp P-1 or Trp P-2 (supplied by the Cancer Research Resources Project of the Ministry of Education, Science and Culture, Japan) was activated by incubating in the S-9 mix at 37°C for various periods of time. The S-9 mix contained 8 \( \mu \)mol MgCl\(_2\), 33 \( \mu \)mol KCl, 5 \( \mu \)mol glucose-6-phosphate, 4 \( \mu \)mol NADPH, 4 \( \mu \)mol NADH, 100 \( \mu \)mol phosphate buffer, pH 7.4, and desired concentrations of S-9 from polychlorinated biphenyl-treated rat or Syrian hamster livers, in total of 1 ml.\textsuperscript{17} Optimal or near-optimal conditions of mutagen activation were biologically determined by measuring the maximum induction of SCE in Chinese hamster cells\textsuperscript{18,19}, which turned out to be 2\% rat-liver S-9 mix for 15 min for AFB1, and 4\% rat-liver or 2\% hamster-liver S-9 mix for 15–30 min for Trp P-1 and Trp P-2\textsuperscript{19} (data not shown).

Exponentially growing cells were treated with optimally activated AFB1, Trp P-1 or Trp P-2 in phosphate-buffered saline for 1 h at 37°C. After two washes, clone survival\textsuperscript{13,20}, autoradiographic unscheduled DNA synthesis (UDS)\textsuperscript{20}, and SCE\textsuperscript{16,20} were assayed as described in the respective literatures. Accumulation of single-strand breaks (SSBs) after a 4 h incubation of mutagen-treated cells with 10 \( \mu \)M arabinofuranosyl cytosine (araC)-plus-2 mM hydroxyurea (HU) was also determined for the presence of excision breaks by 5–20\% alkaline sucrose gradient centrifugation, as described previously\textsuperscript{20} and in Table 2.

RESULTS AND DISCUSSION

AFB1 optimally activated with 2\% rat-liver S-9 mix for 15 min exhibited a 5-fold more lethal effect on XP2BI cells [Do (mean lethal dose that reduces survival in the exponential region to 37\%) = 0.07 \( \mu \)g/ml] than that on NHSF46
Fig. 1. Clone survival curves of NHSF46 and XP2BI group G cells. Exponentially growing cells were treated for 1 h with optimally activated mutagens and clone-survival was determined after an incubation for 14 days. (A) AFB1 activated with 2% rat-liver S-9 mix for 15 min (closed symbols) or not activated (open symbols), (B) Trp P-1 activated with 4% rat-liver S-9 mix for 30 min (closed symbols) or not activated (open symbols), (C) Trp P-2 activated with 4% rat-liver S-9 mix for 30 min (closed symbols) or not activated (open symbols). ○ ●, NHSF46; □ ■, XP2BI.

Table 1. Comparison of mean lethal dose (Do)

| Mutagen | Metabolic activation | Do, µg/mla | Do ratio (NHSF46/XP2BI) |
|---------|----------------------|-------------|------------------------|
|         | S-9 mix | Incubation time, min | NHSF46 | XP2BI |                  |
| AFB1    | None    | 15           | 6.00      | 3.20    | 2                  |
|         | 2% rat  | 15           | 0.34      | 0.07    | 5                  |
|         | 20% ratb | 30          | 1.40      | 0.60    | 2                  |
| Trp P-1 | None    | 30           | 8.0       | 3.9     | 2                  |
|         | 4% rat  | 30           | 6.0       | 2.2     | 3                  |
| Trp P-2 | None    | 30           | 10.8      | 3.2     | 3                  |
|         | 4% rat  | 30           | 3.2       | 1.4     | 2                  |

a. Do alone was compared since extrapolation number equalled 1 (Fig. 1).
b. Not shown in Fig. 1. Over-metabolism of AFB1.
normal cells (Do = 0.34 µg/ml) (Fig. 1a, Table 1). However, AFB1 over-metabolized (20% rat-liver S-9, 30 min) produced a greatly diminished lethal effect on both strains (Table 1), implying a greater inactivation of reactive AFB1 metabolite. Trp P-1 optimally activated with 4% rat-liver S-9 mix for 30 min induced a more effective killing of XP2BI group G cells (Do = 2.2 µg/ml) than that of NHSF46 cells (Do = 6.0 µg/ml) (Fig. 1b, Table 1). Similarly activated Trp P-2 was twice more effective in inactivating NHSF46 and XP2BI cells than Trp P-1 (Fig. 1c, Table 1). Without the S-9 activation, AFB1, Trp P-1 or Trp P-2 effected a very small similar lethality by itself, with overall Do values of 9.0-10.8 µg/ml for NHSF46 and 3.2-3.9 µg/ml for XP2BI (Table 1), thereby suggesting a minor activity of cellular metabolism.

Fig. 2 indicates inductions of UDS in NHSF46 and XP2BI cells treated for 1 h with optimally activated AFB1, Trp P-1 or Trp P-2. All the agents induced UDS in NHSF46 cells, whose increase depended on the mutagen concentration up to 20 µg/ml. Beyond it, induced UDS was rather decreased due to inhibition of repair. On the other hand, a very little, similar induction of UDS in XP2BI cells by all the agents indicates the inability of the cells to repair DNA damage by nucleotide excision repair.

Table 2 shows the araC/HU-induced accumulation of SSBs during a 4 h post-incubation period. Without araC/HU, we detected no significant SSBs even in mutagen-treated NHSF46 cells (data not shown), as described previously.

Fig. 2. Induction of UDS in NHSF46 and XP2BI cells. Cells were treated for 1 h with AFB1, Trp P-1 or Trp P-2 optimally activated as indicated, and incubated for 4 h with 5 µCi/ml of [3H-methyl]thymidine (5 Ci/mmole) in the presence of 2 mM HU, followed by autoradiography.
with UV.) In the presence of araC/HU, optimally activated AFB1 induced more SSBs, approximately 1 SSB/10^9 daltons per 1 µg/ml, in repair-proficient NHSF46 cells than Trp P-1 or Trp P-2, which showed 1/2 or less accumulation on the same basis. This indicates that NHSF46 cells are proficient in excision repair of damage induced by these agents. XP2BI cells, that are totally defective in the initial incision of UV-irradiated DNA, 12, 13) exhibited a little accumulation of araC/HU induced SSBs, that is, roughly 20% of the normal cell level, after treatment with any agent.

Table 2. AraC/HU-induced accumulation of excision SSBs.

| Cell strain | Mutagens | Mn_{b} \times 10^{-6} | SSBs per 10^9 dalton | SSBs/10^9 dalton per 1 µg/ml | % of NHSF46 |
|-------------|-----------|------------------------|----------------------|-----------------------------|------------|
| NHSF46      | None      | 124                    | -                    | -                           | -          |
| AFB1, 5 µg/ml | 77        | 4.92                   | -                    | 0.98                        | -          |
| Trp P-1, 10 µg/ml | 92     | 2.81                   | 1.04                 | 0.10                        | 26         |
| Trp P-2, 10 µg/ml | 84     | 3.84                   | -                    | -                           | -          |
| XP2BI       | None      | 124                    | -                    | -                           | -          |
| AFB1, 5 µg/ml | 112      | 0.86                   | -                    | 0.17                        | 17         |
| Trp P-1, 10 µg/ml | 115     | 0.63                   | 1.04                 | 0.10                        | 21         |
| Trp P-2, 10 µg/ml | 110    | 1.04                   | -                    | -                           | -          |

a. AFB1 was optimally activated with 2% rat-liver S-9 mix for 15 min, and Trp P-1 and Trp P-2 with 2% hamster S-9 mix for 15 min. Cells were treated with mutagens for 1 h and incubated further in 10 µM araC and 2 mM HU for 4 h. Cells were lysed and DNA was centrifuged at 35,000 rpm for 1 h through 5–20% alkaline sucrose gradient using an SW50.1 rotor of a Beckman model L5-50 ultracentrifuge.

b. Number average molecular weight. Standard error fell within 10% of mean.

c. From the calculation of SSBs/dalton.

The above results suggest that DNA damage induced by AFB1, Trp P-1 or Trp P-2 is repairable by nucleotide excision repair. This situation is similar to the repair of UV, 12, 13) 4-nitroquinoline-1-oxide, 21, 22) or acetylaminofluorene 23) lesions in human cells. Defective repair of AFB1 damage in the present XP2BI group G cells is consistent with the previous results of Sarasin et al. 11) with XP group A cells. A more advanced analysis of AFB1 adducts and of specific repair in human cells has revealed that the major primary DNA-adduct in vivo is 2,3-dihydro-2-(N'-guanyl)-3-hydroxy-AFB1 (AFB1-N'G), 24) which is removed spontaneously and enzymatically from normal human cells and only spontaneously in XP group A cells. 24) The secondary product, AFB1-triamino-Py formed from AFB1-N'G, is a long-persisting lesion in both normal and XP cells. 24) In consequence, since XP cells are defective in the enzymatic excision of AFB1-
N'-G, the amount of AFB1-triamino-Py remaining 48 h post-treatment is higher in XP cells than in normal cells. Thus, AFB1-N'-G may be a primary lesion responsible for the repair in normal cells and enhanced killing of XP cells, as observed in this study.

N-hydroxy-Trp P-2 forms covalent DNA-adducts which are reparable in excision-proficient normal human cells, but not in XP group G (see above) and group A cells (our unpublished results). Activated Trp P-1 appears to be less effective in inducing killing (Fig. 1), UDS (Fig. 2) and excision breaks (Table 2) in normal cells than does Trp P-2. This is consistent with the stronger mutagenicity of Trp P-2 to Salmonella compared to Trp P-1. Adduct species produced by tryptophan pyrolysate and repair remain to be studied in further detail. About 20% accumulation of araC/HU-induced SSBs in treated XP2BI cells compared to NHSF46 cells (Table 2) may suggest a possible involvement of base

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**Fig. 3.** Induction of SCE.
NHSF46 and XP2BI cells treated for 1 h with activated AFB1, Trp P-1 or Trp P-2, and incubated for 72 hr in the medium containing 10 μM 5-bromo-2′-deoxyuridine and 10 μM deoxycytidine, followed by the Hoechst 33258-Giemsa differential staining of chromatid. Conditions of metabolic activation were indicated in the figure. (A) Optimally activated AFB1. SCE rates: 8 and 14 SCEs/cell per 0.1 μg/ml for NHSF46 and XP2BI respectively. (B) Over-metabolized AFB1. SCE rates: 7 and 13 SCEs/cell per 1 μg/ml for NHSF46 and XP2BI respectively. (C) Trp P-1. SCE rate: 5 SCEs/cell per 1 μg/ml for both strains. (D) Trp P-2. SCE rate: 4—5 SCEs/cell per 1 μg/ml for both strains.
excision repair or apurinic site endonuclease repair following spontaneous depurination.

Fig. 3a displays a twice higher SCE induction by optimally activated AFB1 in XP2BI cells (rate: 14 SCEs/cell per 0.1 µg/ml) than in NHSF46 cells (rate: 8 SCEs/cell per 0.1 µg/ml). Proportional induction of SCE by activated AFB1 in terms of the XP2BI/NHSF46 ratio (= 2) is less than that of cell inactivation (NHSF46/XP2BI = 5, as shown in Table 1). Thus, AFB1 damage is not well manifested as SCE. Over-metabolism (20% S-9, 30 min) of AFB1 caused a 10-fold less induction of SCE, since the SCE rates were 13 and 7 SCEs/cell per 1 µg/ml for XP2BI and NHSF46 cells respectively (Fig. 3b), agreeing with a less cell-inactivation as shown in Table 1.

Trp P-1 (Fig. 3c) and Trp P-2 (Fig. 3d), when optimally activated with 4% rat-liver or 2% hamster-liver S-9 mix, induced an indistinguishable rate of 4–5 SCEs/cell per 1 µg/ml between NHSF46 and XP2BI cells. Although the SCE mechanism is not well understood, its induction by Trp P-1 or Trp P-2 is not well correlated with defective repair of induced DNA lesions.

In conclusion, activated AFB1, Trp P-1 or Trp P-2 induces DNA damage which is repaired by nucleotide excision repair, and XP cells are defective in its repair.

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