CHLORAMPHENICOL DAMAGES BACTERIAL DNA

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SUMMARY L(+)-threo-chloramphenicol induces reversion of His⁻ Salmonella typhimurium strains TA100 and TA1535 in the conventional Ames' assay without microsomal activation. Any mutagenicity of D(-)-threo-chloramphenicol was masked by toxicity. Similarly, a sensitive fluctuation test showed mutagenesis with L(+)-threo-chloramphenicol at concentrations of 0.5 μM and above but the D(-) isomer proved to be toxic even at these low levels. The L(+) isomer caused single strand breaks in the DNA of Escherichia coli B/r and Salmonella typhimurium strains TA1535, TA100 and TA1976. The D(-) isomer caused breaks in Escherichia coli B/r and Salmonella typhimurium TA1976 although it was less effective and it did not produce DNA breaks in TA1535 or TA100.

D(-)-threo-chloramphenicol is known to cause aplastic anaemia in a small fraction of the people to whom it is administered (1), and may be leukemogenic (2,3). Attempts to investigate the mutagenicity of D(-)CAP* toward bacteria have been foiled by its toxicity (4) which in turn is a consequence of inhibition of protein synthesis. The L(+) isomer, however, does not inhibit protein synthesis (5) and is therefore much less toxic than the D(-) form. In this paper we show that L(+)CAP is mutagenic and that both D(-)CAP and L(+)CAP cause breaks in DNA.

MATERIALS AND METHODS

Chemicals: L(+)-threo-chloramphenicol and D(-)-threo-chloramphenicol were gifts from Dr. H.E. Machamer of Parke-Davis, Detroit, MI. The L(+)-ρ-methyl sulphonyl and L(+)-ρ-methylthio analogues of chloramphenicol were gifts from Dr. F.C. Nachod, Sterling-Winthrop Research Institute, Rensselaer, NY. Stock solutions of the drugs were prepared in DMSO. The final concentration of DMSO in top agar or liquid medium never exceeded 1%. [Methyl-^{3}H] thymidine (19 Ci/mmol) was purchased from Amersham/Searle, Arlington Heights, IL.

Bacteria and Mutagenicity Assays: The following Salmonella typhimurium strains were obtained from Dr. B.N. Ames: TA100 and TA98 (plasmid-containing, uvr B)(6); TA1535 (uvr B)(7); TA1976 (uvr$^+$)(7). The cultures used were frozen in Penassay nutrient broth at -90°C. Escherichia coli B/r (uvr$^+$) was obtained from Atomic Energy of Canada Ltd., Chalk River, Ontario. Reversion

* Abbreviations: D(-)-threo-chloramphenicol, D(-)CAP; L(+)-threo-chloramphenicol, L(+)CAP.

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of His^- to His^+ in strains TA100, TA98 and TA1535 was determined using the method of Ames et al (7). For increased sensitivity the modified fluctuation assay of Green et al (8) using strain TA100 was employed. Fifty small tubes each containing 2 ml of Davis-Mingioli medium (8), histidine (0.5 µg), biotin (0.83 µg), glucose (0.4%), bromcresol purple (0.1 µg) and 10^3 His^- bacteria were incubated for 4 days at 37°C. Positive tubes were those in which a turbid culture of His^+ revertants had changed the indicator from purple to yellow.

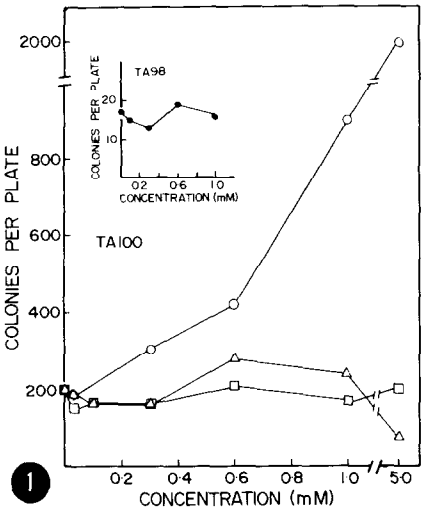
Determination of Breaks in DNA: Exponentially-growing bacteria were labelled with 15 µCi [Methyl-^{3}H]thymidine per ml at 37°C for 1 h in the case of E. coli B/r and for 2 h in the case of S. typhimurium TA1535, TA100 and TA1976. The cells were spun down, resuspended in Penassay broth with chloramphenicol for 30 min and then lysed and subjected to centrifugation on alkaline sucrose gradients as described previously (9,10).

RESULTS

L(+)CAP at concentrations of 0.6 to 5 mM induced His^+ revertants in strains TA100 (Fig. 1) and TA1535 (Fig. 2) but not in TA98 (Fig.1). With the modified fluctuation test (which is 100-1000 times more sensitive than plate tests (8)) the mutagenicity of L(+)CAP could be detected at concentrations as low as 0.5 µM (Table 1). Concentrations up to 200 µM gave progressively larger numbers of positive tubes (Table 1) but 1000 µM proved to be toxic (all tubes remained negative). The L(+)-*p*-methylsulphonyl and L(+)-*p*-methylthio analogues of L(+)CAP, compounds which lack the *p*-nitro group, failed to induce revertants in plate tests using TA100 and TA1535 (Fig.1 & 2) and TA98 (not shown).

With D(-)CAP, no induced His^+ revertants could be detected. On plates this agent was toxic at 0.02 mM - a concentration which would not have given a positive response with L(+)CAP. Likewise, in the fluctuation test (Table 1), D(-)CAP did not produce a statistically significant increase in the frequency of revertants.

DNA breaks were found in E. coli B/r (Fig. 3) and S. typhimurium TA1976 (Fig. 4) exposed to either D(-)CAP or L(+)CAP although D(-)CAP was somewhat less effective. In the uvr B strains of S. typhimurium (TA1535,Fig 5, and TA100 (not shown)) L(+)CAP caused breakage but the effect was consistently somewhat smaller than with uvr^+ strains (S. typhimurium TA1976 and E. coli B/r). Little breakage of DNA by D(+)CAP was detected in the uvr B strains



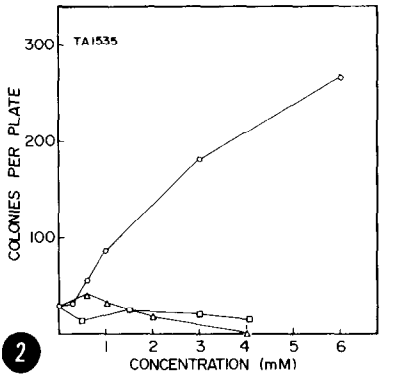


Fig. 1. Results of mutagenicity tests in S. typhimurium strains TA100 and TA98 using L(+)-chloramphenicol or two of its analogues. The drug concentrations indicated are those in the 2 ml of agar overlay. See text for additional details. Symbols: —○—, L(+)CAP; —□—, L(+)-p-methyl sulphonyl analogue; —△—, L(+)-p-methylthio analogue. The insert shows results obtained using strain TA98 with L(+)CAP.

Fig. 2. Results of mutagenicity tests in S. typhimurium strain TA1535 using L(+)-chloramphenicol or two of its analogues. See caption to Fig. 1 for details.

(Fig.5). Neither the D nor L isomers of the p-methylsulphonyl and p-methylthio analogues at 5 mM caused DNA breaks in E. coli (results not shown).

DISCUSSION

McCann et al (4) found that the toxicity of D(-)CAP interfered with attempts to examine the mutagenicity of this drug in S. typhimurium. Our results show that L(+)CAP (which does not inhibit bacterial protein synthesis (5)) is mutagenic to S. typhimurium TA100 and TA1535 but not TA98. These results indicate that L(+)CAP damages DNA, causing base substitutions but not frameshifts (7). It is of interest that the concentrations of drug found in the serum of humans receiving D(-)CAP as an antibiotic were several times greater than those required to induce mutations (11).

Table 1. Results of the fluctuation assays for mutagenicity using L(+)-chloramphenicol and D(-)-chloramphenicol in Salmonella typhimurium TA100. See Methods for experimental details.

Drug	Conc. (μM)	Number of expts.	Average no. of positive tubes	Significance
Control	0	5	18.4	
L(+)CAP	1000	1	0	*
	200	1	50	P<0.01
	20	2	47	P<0.01
	2	4	32.7	P<0.01
	1	2	30.5	P<0.05
	0.7	2	34	P<0.01
	0.5	2	29	P<0.05
D(-)CAP	2	2	0	*
	1	4	16.2	NS**
	0.7	2	15	NS
	0.5	4	23.7	NS
	0.3	2	25	NS
	0.1	2	20	NS

* These results are probably due to toxicity.

** Not significant to the 5% confidence level.

Since both isomers caused DNA breaks, it seems likely that D(-)CAP produced essentially the same molecular damage to DNA as the L(+) isomer but that toxicity masks its mutagenic effect even at the low concentrations used in the fluctuation assay. The reason that the DNA breaking potency of D(+)CAP is lower than that of the L(-) isomer is not clear but may be a result of inherently lower uptake, activation or activity of the D form or could indicate that continued protein synthesis is required for maximum breakage to occur. Also, the fact that we obtained no really convincing evidence that D(+)CAP induced breaks in uvr B bacteria could indicate that, in these strains, the damage produced by D(+)CAP was below the detectable threshold while the somewhat greater damage induced by L(+)CAP was still easily

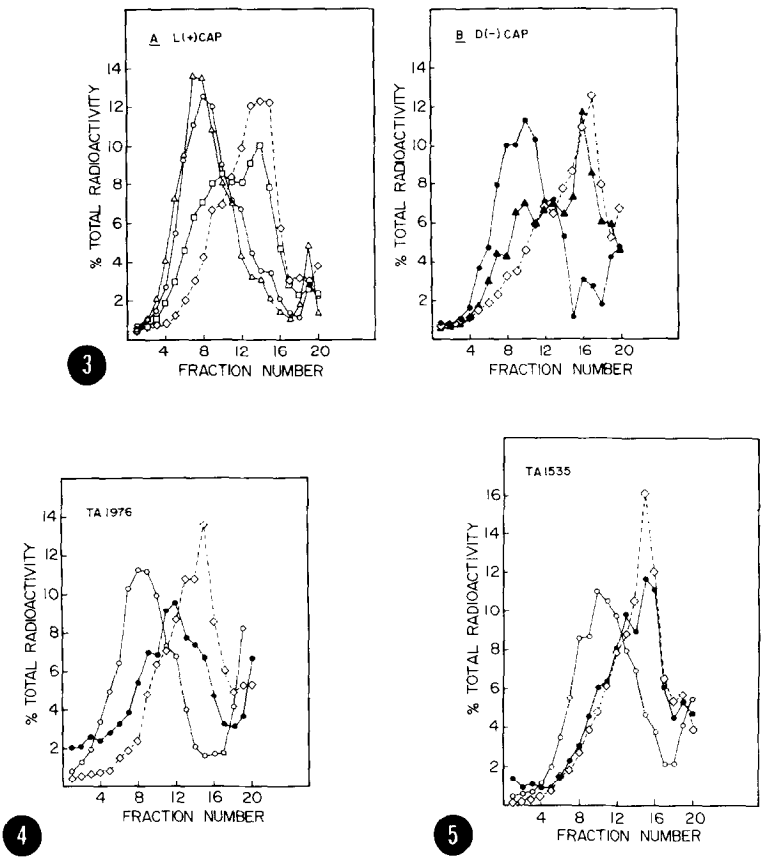


Fig. 3. Alkaline sucrose density gradient analysis of DNA from E. coli B/r exposed to (A) L(+)-chloramphenicol or (B) D(-)-chloramphenicol. Sedimentation was from left to right. Symbols:--◇--, control; —□—, 0.5 mM L(+)CAP; —△—, 2 mM L(+)CAP; —○—, 5 mM L(+)CAP; —▲—, 2 mM D(-)CAP; —●—, 5 mM D(-)CAP.

Fig. 4. Alkaline sucrose density gradient analysis of DNA from S. typhimurium TA1976 exposed to the L(+) or D(-) isomers of chloramphenicol. Sedimentation was from left to right. Symbols:--◇--, control; —○—, L(+)CAP at 5 mM; —●—, D(-)CAP at 5 mM.

Fig. 5. Alkaline sucrose density gradient analysis of DNA from S. typhimurium TA1535 exposed to L(+)- or D(-)-chloramphenicol. See caption to Fig. 4 for details.

detectable. Alternatively, this result could indicate that the uvr^+ gene must be present before lesions produced by D(+)CAP are converted into breaks. The DNA of E. coli B/r seems to be more sensitive than that of the S.

typhimurium strains to both isomers of chloramphenicol.

It is well established that the mutagenicity of nitroheterocyclic compounds is dependent on the presence of the nitro group (12,13,14) which, in a wide variety of organisms, is reduced to yield highly reactive intermediates that damage DNA (13). From our negative results with the two analogues (L(+)-*p*-methylsulphonyl and L(+)-*p*-methylthio chloramphenicol), it seems probable that the nitro group of chloramphenicol is required for mutagenicity and DNA damage. "Activation" of the nitro group of CAP may be the result of the action of CAP reductases which occur in bacteria and animal cells (15).

Lark (16) and Pato (17) have demonstrated that D(-)CAP slows bacterial DNA replication and postulate that this is due to the interference of D(-)CAP with protein synthesis thereby affecting the proteins involved in the initiation of replication. However, it is possible that part of the slowdown of replication could be a consequence of damage to DNA.

While the other known biochemical effects of D(-)CAP, inhibition of bacterial and mitochondrial protein synthesis (18) and inhibition of the respiratory chain between NADH and cytochrome b (19), undoubtedly account for many of the biological effects of this drug, we suggest that there may be other situations in which DNA damage is important. Chloramphenicol inhibits DNA synthesis in mammalian cells but the analogues tested here do not (20,21). In this connection it is of interest that the D(-)-*p*-methylsulphonyl analogue of chloramphenicol does not cause aplastic anaemia (22). From these results and those presented here, it seems possible that DNA damage could be involved in the induction of aplasia. Finally, the observation that D(-)CAP damages DNA suggests that the potential carcinogenicity of this compound should be taken seriously.

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