Mar. Univ. Ecz. Der.,
11 (1 - 2), 141 - 150 (1995)

J. Pharm. Univ. Mar.,
11 (1 - 2), 141 - 150 (1995)

PRECLINICAL ANTITUMOR ACTIVITY OF A NOVEL OLIVACINE DERIVATIVE: S 16020-2 (NSC-659687)

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ABSTRACT

Structural modifications to develop olivacine analogs with reduced cross-resistance and side effects resulted in the discovery of S 16020-2. This drug showed a very potent cytotoxic effet in vitro and a high therapeutic antitumor activity in vivo. 60 % of P388-bearing mice were cured following the administration of 60 mg/kg/day (with an intermittent schedule) x3. The drug was still significantly active against the resistant P388/VCR model while adriamycin was totally inactive. Three intermittent injections of 40 mg/kg induced 100 % cure in established Lewis lung carcinoma. Xenografts of human H-69 small cell lung carcinoma and H460 non small cell lung carcinoma were significantly sensitive to S 16020-2. These properties deserve further pharmacotoxicological studies.

INTRODUCTION

Ellipticine and its analogs are known to induce DNA damage, chromosomal aberations and sister chromatid associated with cytotoxicity in target tumor cells (Pommier et al., 1985 ; Auclair et al. 1987 ; Pommier et al., 1988 ; Noviello et al., 1994) probably acting as topoisomerase II poisons (Pommier et al., 1985).

Ellipticine is a weak DNA binder and the addition of substituants such as 9-hydroxy group increases both DNA binding, G.C. base-pair selectivity of binding and in vitro cytotoxicity (Auclair 1987, Schwaller et al., 1989). Non-intercalating analogs of ellipticine are inactive as antitumor agents, supporting the hypothesis that intercalation is necessary but not sufficient for antitumor activity. Extension of ellipticine series by the synthesis of various heterocyclic derivatives bearing [(dialkylamino)alkyl]amino side chains led to a number of new derivatives which display high antitumor properties in experimental models (Nguyen et al., 1987 ; Bisagni et al., 1988 ; Atassi et al., 1989 ; Nguyen et al., 1992). In all cases, the basic side chains seem to play a key role, either to increase or to confere the antitumor properties. This observation prompted Bisagni's group to undertake the synthesis of the 1-(N-substituted carbamoyl)-9-methoxy-(and 9-hydroxy)-6H-pyrido[4,3-b]carbazoles of the biological active chromophore olivacine (Jasztold-Howorko et al., 1994).

This structural modification to develop olivacine analogs with reduced cross-resistance and less side effects resulted in the discovery of S 16020-2 or 5,6-dimethyl-9-hydroxy-1 (N,N-dimethylamino-ethylaminocarbonyl)-6Hpyrido[4,3-b]carbazole dichloro hydrate (fig.1).

This paper reports the antitumor properties of S 16020-2 both in vitro and in vivo tumor models including resistant cell lines, a murine solid tumor and human tumor xenografts in nude mice.

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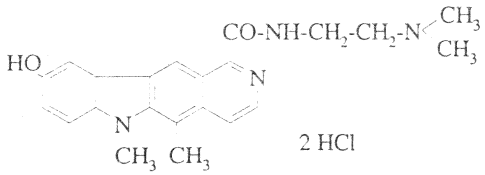


FIG. 1 : Chemical structure of S 16020

MATERIAL AND METHODS

Drugs

Adriamycin was purchased from Farmitalia (France) and elliptinium acetate from Pasteur Vaccins (France). S 16020-2 was synthesized by E. BISAGNI's group (Jasztold-Howorko et al., 1994). For in vitro tests, the 3 compounds were solubilized at 10^{-2}M in distilled water, aliquoted and stored at -20°C. The solutions were thawed just before the experiments. For in vivo studies, the drugs were solubilized in distilled water and diluted with saline. The drugs were administered in 0.1 ml per 10 g animal weight at the doses indicated in the figures.

Cell lines and proliferation assays (MTA)

NCI-H460, a non small cell lung carcinoma from human origin, and P388, murine lymphocytic leukemia, cell lines were obtained from NCI. The multidrug resistant subline P388/VCR-20, which resistance was induced in our laboratories, is described in Pierré et al., 1992 ; Léonce et al., 1992.
Cells were cultivated in RPMI 1640 medium supplemented with 10 % of complemented fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin and 10 mM Hepes, pH = 7.4. Cells were grown in this complete culture medium at 37°C in 5 % CO_2 / 95 % air. All medium and supplements were from Gibco (France).
The MTA (microculture tetrazolium assay) is a colorimetric assay based on the reduction of a tetrazolium salt by the mitochondrial enzymes of the respiratory chain. For details on its application, refer to Aley et al., 1988 ; Pierré et al., 1991. The percentage of growth was calculated as IC50 (concentration of drug inhibiting the growth of malignant cells by 50 %).

Mice and in vivo tumor models

Female B6D2F1 (C57Bl/6 x DBA2) , inbred DBA2 (P388 leukemia) and C57Bl6 (Lewis lung carcinoma, LLC) were used in murine tumor models. Nude femal athymic mice congenic of swiss strain homozygous for the nude gene (nu/nu) were used for NCI-H460 non small cell lung carcinoma xenograft, NSCLC and NCI-H69 a small cell lung carcinoma xenograft, SCLC. All mice were purchased from Iffa Credo (France). The conditions of the experiments as well as the doses and schedule of treatment are indicated in the results. The tumors were measured twice a week in two dimensions. Volume of sc tumors was calculated according to the formula 0.5 x length x width^2. The Vt/V0 value was determined for all individual tumors. V0 is the initial volume and Vt is the volume at the indicated time t. The median Vt/V0 values for each group of mice at each measurement time were used to draw the tumor growth curve. Needless to say that regression exists when V0 is greater than Vt.

RESULTS

Inhibition of cellular proliferation

Fig. 2 shows the inhibition of H460 cell proliferation by S 16020-2 in comparison with adriamycin (ADR) and elliptinium acetate (ELP).
The dose response curve was obtained after addition of the compounds to tumor cells in the exponentional phase of growth. The cells were incubated at 37°C for 4 doubling times. After adding 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma), the cells were incubated for 4 hours at 37°C. The plates were then centrifuged (1600xg, 10 min), the supernatant aspirated and the formazan solubilized by 100 µl of DMSO under mild shaking for 30 sec. The optical density was read at 540 nM with a plate reader (Multiskan MCC, Labsystem) connected to a computer.
S 16020-2 appeared as potent as ADR in inhibiting H460 proliferation. The cell lines whose resistance was induced by VCR, the P388/VCR-20, appeared to be resistant to ADR but not to S 16020-2. Moreover, this cell line, which display the classical multidrug resistance (MDR) phenotype appeared as sensitive to S 16020-2 as its sensitive parental counterparts (fig. 3).

In vivo antitumor activity

Leukemia

The P388 lymphocytic leukemia (sensitive line) and the resistant P388/VCR-20 subline were inoculated (10^6 cells per mouse) intraperitoneally (IP) to BDF1 mice on day 0. S 16020-2 was administered intraveinously (IV) on days 1,5,9. Fig. 4 shows the percentage of survival in treated animals bearing the sensitive P388 leukemia in comparison with the survival of the control group. ADR was used under the same experimental conditions as positive controls. S 16020-2 showed an important antitumor effect at all the doses used ranging from 10 mg/kg/day to 80 mg/kg/day, 20 % and 60 % of cures were observed at 40 and 60 mg/kg/day, respectively. The wider range between the minimum effective dose and the optimal dose observed for S 16020-2 indicates the higher therapeutic index of this drug. The P388/VCR-20 leukemia, in which the resistance was induced by VCR, was totally resistant to VCR and ADR after an IV administration on days 1,5,9 (fig. 5) while S 16020-2 retained a good antitumor effect but no long term survivors were registered in this model.

Lung carcinomas

1) Lewis lung carcinoma (LLC)

To evaluate the antitumor effect of S 16020-2 in this highly metastatic tumor model, small fragments of the tumor were implanted subcutaneously (SC) on day 0 in BDF1 mice. S 16020-2 was administered IV on days 3,6,9 while endoxan was administered IP following the same schedule. The 2 drugs were active and induced at their optimal doses, a high increase in life span of treated mice. However, S 16020-2 was able to induce 100 % cure at 40 mg/kg/day (fig. 6).

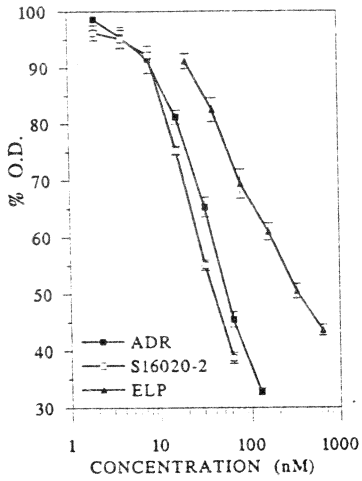


FIG. 2: Inhibition of H460 cells growth by S 16020-2 (□).
It is determined by a proliferation inhibition assay expressed as percentage of untreated controls. Cytotoxicity of adriamycin (ADR) (■) and elliptinium acetate (ELP) (▲) is represented.

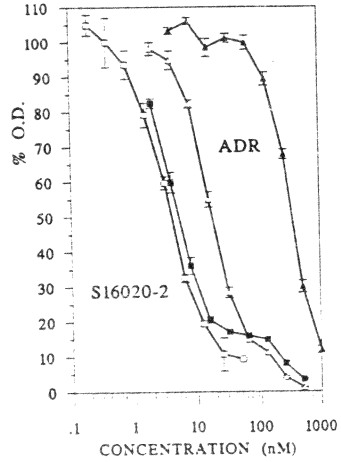


FIG. 3 : Inhibition of P388 sensitive (□, Δ) and P388/VCR-20 resistant (■,▲) leukemia cells proliferation by exposure to variant concentrations of S 16020-2 and adriamycin (ADR)

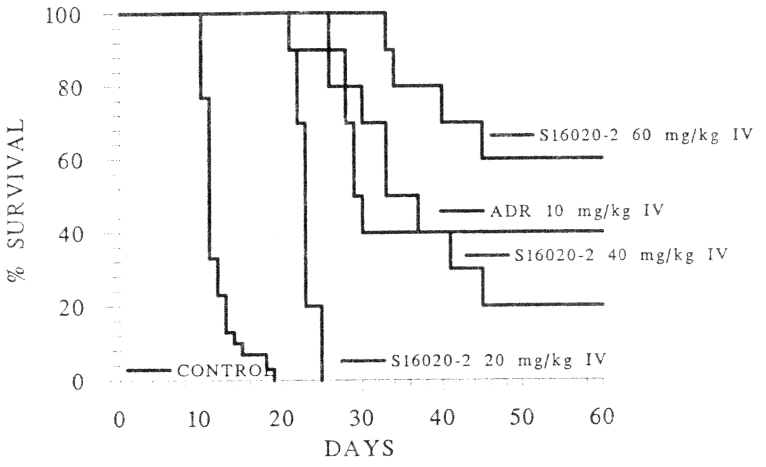
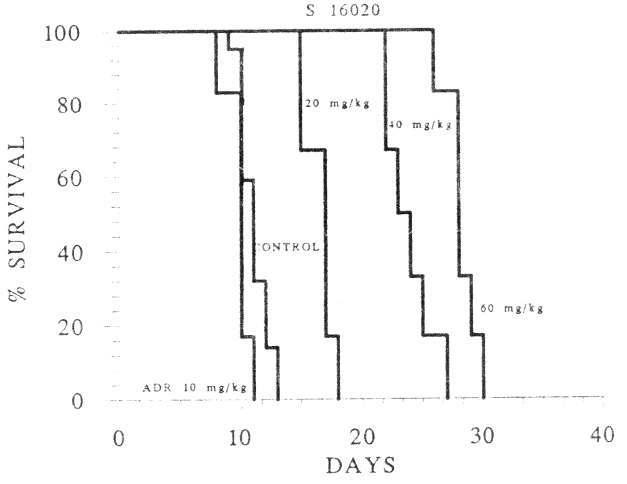


FIG. 4 : Therapeutic effect of S 16020-2 against the P388 sensitive leukemia.

10^6 cells were inoculated on day 0. The drugs were administered IV on days 1,5 and 9 at the doses indicated. The curves represent the percentage of survival mice in treated and control groups as a function of time. Adriamycin (ADR) is used as reference compound.



. IG. 5 : Therapeutic effect of S 16020-2 against the P388/VCR resistant leukemia.

10^6 cells were inoculated in mice in day 0. The drugs were administered IV on days 1,5 and 9. The curves represent the percentage of survival mice in treated and control groups as a function of time.

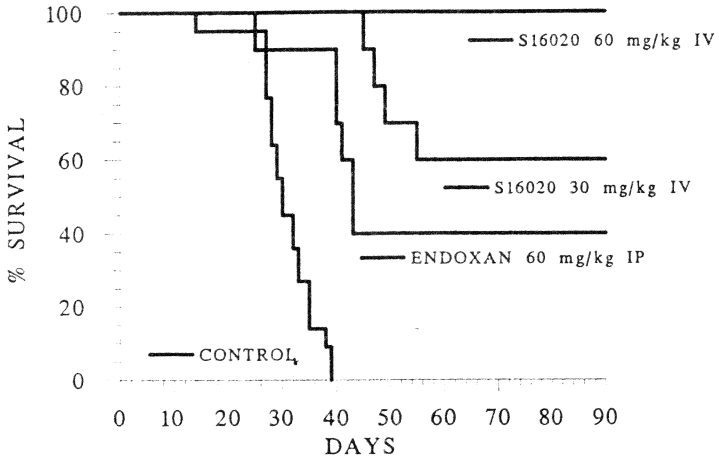


FIG. 6 : Therapeutic effect of S 16020-2 against the Lewis lung carcinoma in mice.

The tumor was implanted SC on day 0 and the drugs were administered on days 3,7 and 11. The IV route was used for S 16020-2 while the IP route was used for reference compound, endoxan. The curves represent the percentage of survival mice in treated and control groups.

2) NCI-H460 non small cell lung carcinoma xenograft

Tumor fragments were transplanted SC bilaterally in the flanks of nude mice by a 10-gauge needle. When the tumor reached an average of 4-6 mm in diameter (considered day 0), S 16020-2 and ADR were administered IV on days 0,7,14. Fig. 7 shows the tumor growth curves of treated groups in comparison with the control group. In the groups of S16020-2 treated mice, we observed an important antitumor effect at 90 and 60 mg/kg/day while ADR, administered at the optimal dose of 10 mg/kg/day appeared less active.

3) NCI-H69 small cell lung carcinoma xenograft

The methodology was the same as for NCI-H460. S 16020-2 showed an important dose-dependent tumor growth inhibition (fig. 8). In this experiment, S 16020-2 at 60 mg/kg/day showed the same activity obtained by ADR at the optimal dose of 9 mg/kg/day while the optimal dose of S 16020-2 was the most active treatment.

DISCUSSION

Under the experimental conditions used (continuous exposure of H460 and P388 cells), S 16020-2 was a potent cytotoxic compound as potent as ADR, while it was more potent in the P388/VCR-20 cell line, which has a pure classical MDR phenotype. These results suggest that S 16020-2 might retain a good activity on pure classical MDR lines because of its poor recognition by the P-glycoprotein which is super-expressed in the typical MDR lines (the activity against this line was also confirmed in vivo while ADR was totally inactive).
The antitumor effect of S 16020-2 against the murine lung carcinoma LLC and the 2 NCI lung xenografts (H460 (NSCLC) and H69 (SCLC)) may suggest a relative selectivity towards lung carcinomas.
Preliminary results obtained by LE MEE et al. 1994 showed that S 16020-2 was able to intercalate into DNA and to interact with topoisomerase II. This result suggests that S 16020-2 shares the same mechanism of action with ADR and other topoisomerase II inhibitors. The observation of a high therapeutic effect of S 16020-2 on advanced murine tumors such as the highly metastatic Lewis lung carcinoma and the important tumor growth inhibition in human lung adenocarcinoma xenografts, may be explained by the formation of very highly stable DNA drug enzyme complex specific sequences of DNA. This explanation is just an hypothesis which need to be demonstrated before describing the real mechanism which induces this high antitumor effect. However, this attractive property of S 16020-2 may deserve further pharmacological and toxicological studies in preparation to an eventual phase I clinical investigation.

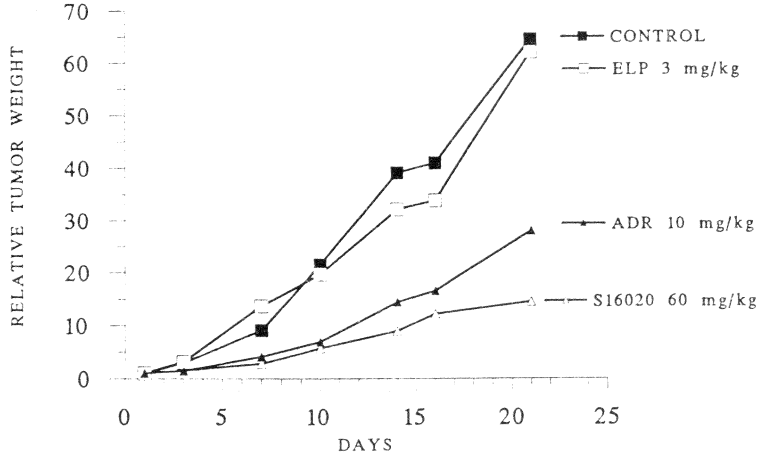


FIG. 7 : Antitumor activity of S 16020-2 against NCI-H460 (NSCLC) human tumor xenograft in nude mice.

Tumor fragments were transplanted SC when the tumors reached an average of 4-6 mm in diameter (day 0). The drugs were administered IV on days 0,7 and 14. The curves represent the tumor growth of treated groups in comparison with the control group.

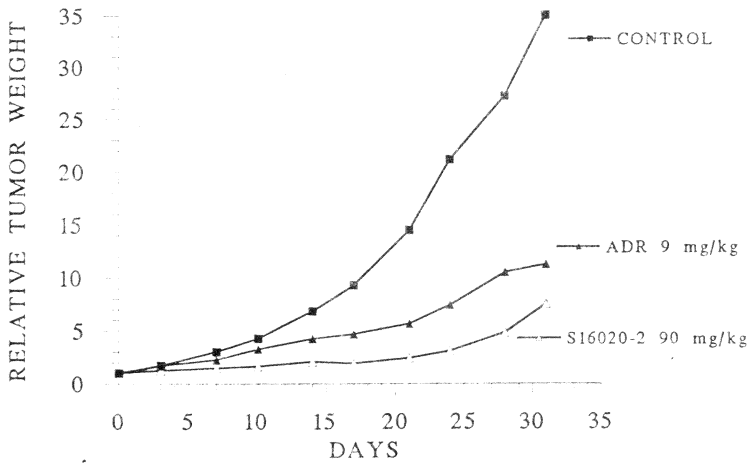


FIG. 8 : Antitumor activity of S 16020-2 against NCI-H69 (SCLC) human tumor xenograft in nude mice.

Curves represent the tumor growth of treated groups in comparison with the control group.

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