Differential regulation of the JNK/AP-1 pathway by S-Adenosylmethionine and methylthioadenosine in primary rat hepatocytes versus HuH7 hepatoma cells

Eduardo Ansorena¹, Carmen Berasain², María J. López Zabalza¹, Matías A. Ávila²,
Elena R. García-Trevijano² and María J. Iraburu¹*

¹Departamento de Bioquímica y Biología Molecular, ²Area de Hepatología y Terapia Génica, CIMA. Universidad de Navarra. 31008 Pamplona, Spain

*To whom correspondence should be addressed

Author’s address: Dr. María J. Iraburu. Departamento de Bioquímica y Biología Molecular. Universidad de Navarra. C/ Irunlarrea 1, 31008 Pamplona, Navarra, Spain.
Tel: 34-48-425600 (Ext. 6481) Fax: 34-48-425619

e-mail address: miraburu@unav.es

Running head: AdoMet and MTA regulation of JNK/AP-1
ABSTRACT

S-adenosylmethionine (AdoMet) and 5’-Methylthioadenosine (MTA) exert a protective action on apoptosis induced by okadaic acid in primary rat hepatocytes, but not in human transformed HuH7 cells. In the present work we analyzed the role played by the JNK/AP-1 pathway in this differential effect. Okadaic acid induced the phosphorylation of JNK and c-Jun and the binding activity of AP-1 in primary hepatocytes, and pretreatment with either AdoMet or MTA prevented those effects. In HuH7 cells pretreatment with either AdoMet or MTA did not affect JNK and c-Jun activation or AP-1 binding induced by okadaic acid. In both cell types, p38 was activated by okadaic acid, but neither AdoMet nor MTA presented a significant effect on its activity. Therefore, the differential effect of both AdoMet and MTA on the JNK/AP-1 pathway could explain their antiapoptotic effect on primary hepatocytes and the lack of protection they show against okadaic acid-induced apoptosis in hepatoma cells.

Key Words: okadaic acid; c-Jun; apoptosis.
INTRODUCTION

Intracellular signalling pathways are involved in the regulation of cell functions, including programmed cell death or apoptosis, and exert their actions in a pleiotropic fashion. JNK, a member of the superfamily of MAPK characteristically activated by stress-stimuli, like UV, TNF-α, or different death receptors is a mediator of apoptosis in primary hepatocytes and other cell types (11,33,31). In several experimental systems the proapoptotic effect of JNK has been shown to depend on the extent and duration of its activation: transient activations are not capable of triggering the apoptotic response and even have an antiapoptotic or proliferative effect, while a sustained activation of JNK leads to apoptosis (12,22,20). The effects of JNK are partly due to the activity of transcription factor AP-1, which is activated by JNK through the phosphorylation of one of its components, the protein c-Jun. Like JNK, AP-1 activity has been related to proliferative or apoptotic responses, depending on the characteristics of the cell type and the stimuli used (2).

Okadaic acid (OA) is an inhibitor of protein phosphatases that presents a proapoptotic effect in hepatocytes and other cell types. Unlike TNF-α or FasL, OA induces apoptosis in the absence of inhibitors of protein synthesis, in a pathway that involves cytochrome c release from the mitochondria and caspase 3 activation (1). On the other hand, OA treatment can induce the phosphorylation and activation of JNK through the inhibition of phosphatases involved in the dephosphorylation of the enzyme (40,17). Some of the effects of OA on gene expression are mediated by the activation of AP-1 (19,36). However, the requirement of gene expression for the apoptotic effect of OA is still a matter of controversy, since in some cell types OA can induce apoptosis in
a protein-synthesis independent way (9), while in others the presence of inhibitors of transcription or translation prevents its apoptotic action (15).

We have previously demonstrated that S-adenosylmethionine (AdoMet) and its derivative 5’-Methylthioadenosine (MTA), two metabolites with wide hepatoprotective actions (3,34,14), prevent the apoptotic effect of OA on primary rat hepatocytes, blocking cytochrome c release and caspase 3 activation induced by this compound. Interestingly, both AdoMet and MTA presented a differential effect in hepatoma cells compared to primary hepatocytes, not being able to protect cells from OA-induced apoptosis (1). In the present work, we evaluate the role played by the JNK/AP-1 pathway in apoptosis of primary rat hepatocytes induced by OA, and its regulation by AdoMet and MTA, both in primary and transformed hepatoma cells.
MATERIALS AND METHODS

Reagents. Okadaic acid (OA) and JNK inhibitor SP600135 were obtained from Calbiochem (San Diego, CA). JNK inhibitor JNKI1 was from Alexis Biochemicals (Lausen, Switzerland). S-adenosylmethionine (AdoMet) was obtained from Boehringer Ingelheim (Ingelheim, Germany). 5’-Methylthioadenosine (MTA) and cycloheximide were purchased from Sigma Chemical Company (St. Louis, MO). Cell culture reagents were from Invitrogen (Carlsbad, CA).

Cell culture and treatments. Freshly isolated hepatocytes were obtained by liver collagenase perfusion from male Wistar rats as described elsewhere (21). Cell viability was determined by measuring trypan blue exclusion. Only hepatocytes showing values above 90% of viability were used for further experiments. Isolated hepatocytes were resuspended and plated in MEM supplemented with 10% foetal bovine serum (FBS), antibiotics and non-essential amino acids. HuH7 cells obtained from American Type Culture Collection (Rockville, MD) were cultured in DMEM medium supplemented with 10% FBS, glutamine and antibiotics. Six hours after plating the cells the medium was replaced by MEM or DMEM supplemented with 1% FBS, and treatments were carried out 12 hours later.

Primary rat hepatocytes and HuH7 cells were treated for the indicated times with either 20 nM or 125 nM OA respectively. When indicated, cells were pretreated with the following compounds: SP600125 (8 hours, 20 or 50 µM), JNKI1 (8 hours, 10 µM), SB203580 (30 min, 10 µM) and cycloheximide (30 min, 100 µM), AdoMet (30 min, 4 mM) or MTA (30 min, 500 µM). The viability of hepatocytes treated with AdoMet 4 mM was evaluated by MTT and found to be similar to that of control hepatocytes (1.12 ± 0.017 versus 1.0).
**Western blot.** Primary rat hepatocytes or HuH7 cells were treated as described above and extracted in Triton buffer. Protein concentration of the resultant samples was determined by BCA and equal amounts of protein were electrophoresed on 12% SDS-polyacrylamide gels and transferred on nitrocellulose membranes (Amersham Biosciences, Piscataway, NJ). Proteins were detected using specific antibodies for caspase 3 (p17 active form), JNK and its phosphorylated form, and phosphorylated p38 purchased from Cell Signaling Technologies (Beverly, MA), or c-Jun, ATF-2 and their phosphorylated forms, and p38 from Santa Cruz Biotechnology (Santa Cruz, CA). After incubation with the secondary antibodies conjugated to horseradish peroxidase (Promega, Madison, WI), bound antibodies were detected by enhanced chemiluminescent system (ECL) from Roche Molecular Biochemicals (Indianapolis, IN).

**Nuclear protein extraction and electrophoretic mobility shift assays (EMSA).** Primary rat hepatocytes or HuH7 cells were treated as described above and nuclear proteins from control and treated cell cultures were obtained by a modification of the method described by Schreiber et al. (32). The consensus sequences for AP-1 and NFκB were obtained from Santa Cruz Biotechnology (Santa Cruz, CA) and labeled with (γ\(^{32}\)P) ATP using T4 polynucleotide kinase (Promega, Madison, WI). The reaction mixtures containing 5 μg of nuclear extracts were incubated with the probe (80,000 cpm) for 30 minutes at room temperature. DNA-protein complexes were separated from unbound probe by electrophoresis on a 6% polyacrylamide gel. Complexes formed were identified by autoradiography of the dried gels. Competition assays were carried out adding a 50x excess of unlabelled probe. For supershift experiments, incubation
mixtures contained the corresponding specific antibodies obtained from Santa Cruz Biotechnology (Santa Cruz, CA).

**Determination of oligonucleosomal (histone-associated) DNA fragments.**

The presence of soluble histone-DNA complexes was measured by the *Cell Death Detection Assay* from Roche Molecular Biochemicals (Indianapolis, IN). For these experiments, primary rat hepatocytes were seeded on 60mm plates at a density of $3 \times 10^6$ cells/dish. Cell death ELISA assays were performed according to the manufacturer’s instructions. Specific enrichment of mono- and oligonucleosomes released into the cytoplasm (enrichment factor, EF) was calculated as the ratio between the absorbance values of the samples obtained from treated and control cells.

**Statistical analysis.** All the experiments were performed at least in triplicate. Data were analyzed using the Kruskal-Wallis test to determine differences between all independent groups. When significant differences were obtained ($p < 0.05$), differences between two groups were tested using the Mann-Whitney U test.
RESULTS

*Okadaic acid activates the JNK/AP-1 pathway in primary rat hepatocytes*

To determine whether the JNK/AP-1 pathway was involved in the apoptotic action of okadaic acid (OA), we first analyzed the phosphorylation levels of JNK in primary hepatocytes treated with OA for different time periods. As shown in figure 1, OA induced the phosphorylation of JNK in a time-sustained fashion, with maximum levels at 4-6 hours of treatment.

Electrophoretic mobility shift assays (EMSA) using a consensus probe for the transcription factor AP-1 showed that the activation of JNK in OA-treated hepatocytes correlated with an increased binding activity of nuclear proteins to AP-1 (Figure 2A). The specific binding of transcription factor AP-1 to the probe was determined by competition studies, adding an excess of non-labelled AP-1 or NFκB probes to the reaction mixture (Figure 2B). To identify the members of the AP-1 family present in the AP-1/DNA complexes, supershift assays were also carried out. As shown in figure 2C, c-Jun and JunB, and lower levels of JunD and Fos, were present in the complexes obtained from hepatocytes treated for 4 hours with OA.

*The JNK/AP-1 pathway is involved in the apoptotic action of okadaic acid on primary rat hepatocytes*

The involvement of the JNK/AP-1 pathway on the apoptotic effect of OA in primary rat hepatocytes was established using the inhibitor for JNK SP600125 (anthra(1,9-cd)pyrazole-6 (2H)-one). This compound does not alter the phosphorylation of JNK but inhibits its kinase activity in a reversible and competitive way (6). Pretreatment of primary rat hepatocytes with SP600125 prevented in a dose-response
fashion the apoptotic effect of OA determined as accumulation of oligonucleosomal fragments on the cytoplasm of the cells. Another pharmacological JNK inhibitor, JNKI1 presented a similar effect. Pretreatment with the protein synthesis inhibitor cycloheximide also caused a significant decrease of OA-induced apoptosis, showing that newly synthesized proteins contribute to the apoptotic response elicited by OA. On the contrary, the inhibitor for p38 (SB203580), a kinase that like JNK is activated by stress-signals, did not prevent apoptosis induced by OA (Figure 3A). The effect of SP600125 was also analyzed on another parameter of apoptosis, caspase 3 activation, which had been shown to be induced by OA in primary rat hepatocytes (1). As expected, JNK inhibition diminished caspase 3 activation in hepatocytes treated with OA (Figure 3B).

AP-1 binding activity was also evaluated in primary hepatocytes that had been pretreated with SP600125 prior to the incubation with OA. The JNK inhibitor effectively prevented the binding of AP-1 to its consensus sequence induced by OA (Figure 3C).

**AdoMet and MTA prevent the apoptotic effect of okadaic acid through the inhibition of the JNK/AP-1 pathway**

Once the role played by the JNK/AP-1 pathway on the apoptotic effect of OA was established we analyzed its regulation by AdoMet and MTA, two compounds that had been previously shown to abolish apoptosis of hepatocytes induced by OA (1). Pretreatment of primary rat hepatocytes with either AdoMet (4 mM) or MTA (500 µM) prevented the phosphorylation of JNK caused by incubation with 20 nM OA for 4 hours, without affecting the total content of the protein. The protein c-Jun, a JNK substrate which was phosphorylated in response to OA, presented lower levels of the
phosphorylated form in cells pretreated with either AdoMet or MTA, while the total protein content was not altered by any of the treatments (Figure 4A). A similar effect was observed using lower doses of AdoMet (1 mM, data not shown).

The phosphorylation levels of p38 and its substrate ATF-2 were also analyzed in hepatocytes treated for 4 hours with OA and pretreated with either AdoMet or MTA. As it was observed for JNK and c-Jun, incubation with OA enhanced the phosphorylated forms of p38 and ATF-2. However, as shown in figure 4A, the phosphorylated forms of p38 and ATF-2 in hepatocytes pretreated with AdoMet or MTA and treated with OA were only slightly lower than those obtained from OA-treated cells.

The effect of AdoMet and MTA in the binding activity of transcription factor AP-1 induced by OA was also determined. AP-1 binding activity was almost totally abolished in extracts obtained from hepatocytes pretreated with AdoMet and MTA and treated with OA in the same conditions than above, as compared to OA-treated samples (Figure 4B).

**AdoMet and MTA have no effect on the JNK/AP-1 pathway activated by okadaic acid in hepatoma cells**

To determine if AdoMet and MTA had different regulatory actions on the JNK/AP-1 pathway activated by OA on tumor cells, we performed in the human hepatoma cell line HuH7 similar experiments to those already described. As observed for primary hepatocytes, treatment of HuH7 cells with OA caused a sustained increase of the phosphorylation levels of JNK (Figure 5A). Pretreatment with the JNK inhibitor SP600125 prevented both caspase 3 activation as well as c-Jun phosphorylation induced by OA on HuH7 cells (Figure 5B).
AP-1 binding activity was also induced in a sustained fashion by treatment with OA, although hepatoma cells presented higher basal levels than hepatocytes (Figure 6A). Supershift experiments were carried out to determine the composition of both basal AP-1, present in untreated cells, and of AP-1 dimers induced by treatment with OA. AP-1 of control samples was mainly composed of c-Jun, and JunD, with lower levels of members of the Fos family (Figure 6B). Extracts from OA-treated cells had a different pattern of AP-1 members, with higher levels of c-Jun, JunB and Fos and lower levels of JunD (Figure 6C).

The effect of AdoMet or MTA on the JNK/AP-1 pathway induced by OA on hepatoma cells was then evaluated. As shown in figure 7A, pretreatment of HuH7 hepatoma cells with AdoMet or MTA did not significantly affect the phosphorylation levels of JNK and c-Jun, which were similar to those observed for OA-treated cells, although some differences between both metabolites were detected, AdoMet having a slight inhibitory effect that was not observed for MTA. The phosphorylation of p38 and ATF-2 induced by OA remained unchanged by pretreatment with either AdoMet or MTA. In the absence of OA, neither AdoMet nor MTA had any effect on the total or phosphorylated protein levels of JNK and c-Jun, p38 or ATF-2 (Figure 7A).

Similar results were obtained in EMSA analysis of AP-1 binding activity in HuH7 cells treated as above: the induction of AP-1 caused by OA was not altered by pretreatment with AdoMet or MTA, and neither compound had an effect in AP-1 binding activity in the absence of OA (Figure 7B).
S-adenosylmethionine (AdoMet) is a metabolite of the essential amino acid methionine involved in cellular transmethylation reactions, and a precursor of polyamine and glutathione synthesis in the liver. AdoMet exerts many hepatoprotective effects that are due to different molecular mechanisms, some of them not related to methylation reactions. As a GSH precursor, AdoMet protects liver cells from oxidative stress caused by ethanol metabolism, xenobiotics or viral infections (25,30). In apoptosis due to the accumulation of toxic bile acids, the protective role of AdoMet is also related to its antioxidant action (7). However, as we have already shown, the protective effect of AdoMet in apoptosis induced by OA in hepatocytes is not caused by its role as GSH precursor, and could be mediated by MTA (1). MTA, which can be formed from decarboxylated AdoMet during polyamine biosynthesis or by the spontaneous splitting of AdoMet under physiological conditions (4), also presents beneficial effects in the liver, including the preservation of hepatocyte viability and the prevention of hepatocarcinoma development (34,28). Interestingly, the synthesis and metabolism of both compounds, AdoMet and MTA, are impaired in chronic liver damage and hepatocarcinoma, and these defects have been associated with the progression of the disease (3,34, 25, 28,8). In the present report, we demonstrate that the antiapoptotic action of AdoMet and MTA in primary hepatocytes is due, at least in part, to their capacity of inhibiting the JNK/AP-1 cascade. The stress-activated kinase p38, also activated by OA, was not significantly regulated by AdoMet and MTA, suggesting that JNK is a specific target for the inhibitory action of both compounds. The JNK/AP-1 pathway is involved in many pathophysiological responses of the liver, including alcoholic liver disease and ischemia/reperfusion injury in liver transplant. In this
respect, recent studies have shown both in culture systems and in animal models for liver transplant, that JNK inhibition prevents apoptosis of hepatocytes and improves survival (37,38,24). JNK activation in the liver is also responsible for insulin resistance and glucose tolerance characteristic of diabetes, and its inhibition exerts beneficial effects in genetic and dietary models of this pathology (27). These data suggest that AdoMet and MTA could be used as JNK inhibitors in pathologies in which JNK activation contributes to cell damage or to the progression of the disease.

Interestingly, AdoMet and MTA have opposite effects depending on the phenotype of the cells: both metabolites present a protective action on apoptosis induced by OA in primary hepatocytes but fail to prevent apoptosis induced by OA in hepatoma cells. Moreover, they also provoke a slight apoptotic effect in the absence of any other apoptotic inducer (1). Therefore, AdoMet and MTA could favor the apoptotic process of malignant cells in two ways: inducing apoptosis of transformed cells by themselves and not preventing the apoptotic action of compounds such as okadaic acid, as they do in non-transformed cells. Our results indicate that both effects are independent: apoptosis induced by AdoMet or MTA on hepatoma cells is not due to the activation of the JNK/AP-1 pathway, since neither of them altered the phosphorylation levels of JNK or the binding activity of AP-1. Other mechanisms, like enhanced expression of the proapoptotic protein bcl-Xs that has been described in HepG2 hepatoma cells, could explain the apoptotic effect of both metabolites in the absence of apoptotic inducers (41).

Our data infers that AdoMet and MTA did not protect HuH7 cells from apoptosis caused by OA because they failed to prevent JNK and AP-1 activation. Previous reports had already described that JNK and c-Jun activation can lead to apoptosis of hepatoma cells (18,16). Moreover, it has been shown that antitumoral
agents exert their effects through JNK activation and apoptosis of hepatoma cells (10,13). Although hepatoma cells present high levels of AP-1 probably related to their proliferative state, an increased AP-1 activity and changes in the specific composition of its components induced by JNK activation would act as negative regulators for cell survival. We found that treatment with OA greatly induced the extent of AP-1 binding activity and also altered the composition of AP-1 components. OA-activated AP-1 had higher levels of JunB and lower levels of JunD than AP-1 from control cells. A decrease of JunD could be involved in apoptosis, since this protein has been described to exert antiapoptotic effects (5,39). On the other hand, an increase in JunB levels could also facilitate apoptosis through inhibition of proliferation by blocking the cell cycle (5, 29).

The lack of effect of AdoMet and MTA on the JNK/AP-1 pathway of hepatoma cells would ensure that these metabolites would not interfere on activation of JNK leading to apoptosis of tumoral cells.

Further studies are needed to establish the molecular mechanism by which AdoMet, and more specifically MTA, which is responsible for the effects of AdoMet in OA-induced apoptosis, prevent the activation of JNK in primary hepatocytes. MTA has been already described to inhibit other kinase activities like tyrosine kinase induced by the fibroblast growth factor receptor and nerve growth factor (35,23,26), or p38 activity induced by LPS (14), but the mechanisms of these effects remain to be elucidated. Nevertheless, the possibility of regulating the JNK/AP-1 pathway in primary but not transformed hepatic cells offers new perspectives for the application of AdoMet and MTA in those pathological processes in which JNK is involved.
ACKNOWLEDGEMENTS

The authors thank José María Mato for critical reading of this manuscript and Eva Petri for technical assistance. ERGT present address: Departamento de Bioquímica, Facultad de Medicina y Odontología, Universidad de Valencia.

GRANTS

This work was supported by Ministerio de Sanidad y Consumo, Fondo de Investigaciones Sanitarias (FIS) grants PI030391, PI020369 and CP04/00123 to MJI, ERGT and MAA respectively. Grant SAF2004-03538 from Ministerio de Educación y Ciencia to MAA. Grants C03/02 and G03/015 from Instituto de Salud Carlos III. Grants ROI AA-12677 from the National Institute on Alcohol Abuse and Alcoholism and R01 AT1576 National Center for Complementary and Alternative Medicine to MAA and ERGT. This work was also supported by the agreement between FIMA and the “UTE project CIMA” and by Gobierno de Navarra.
REFERENCES


FIGURE LEGENDS

Figure 1. JNK phosphorylation in primary rat hepatocytes treated with okadaic acid. Cells were incubated with 20 nM okadaic acid (OA) for the indicated times and JNK was identified in protein extracts by Western blot using specific antibodies for JNK and for the phosphorylated form of the enzyme.

Figure 2. AP-1 binding activity in primary rat hepatocytes treated with okadaic acid. Cells were incubated with 20 nM OA for the indicated times and nuclear extracts obtained as described in Materials and Methods. AP-1 binding activity was determined by EMSA using a consensus probe for the factor. A, Time course analysis of AP-1 binding activity in response to okadaic acid. B, Competition assays for AP-1 binding activity. Cells were incubated with 20 nM OA for 4 hours and a 50x excess of cold probe (AP-1 or NFκB) was added to the reaction mixture. C, Analysis of AP-1 components. Cells were incubated with 20 nM OA for 4 hours and specific antibodies for the indicated members of AP-1 were added to the reaction mixture. The supershifts obtained are indicated with arrow-heads.

Figure 3. Effect of inhibitors on apoptosis of primary hepatocytes caused by OA. A, Effect of SP600125 (SP), JNKI1, SB203580 (SB) and cycloheximide (CHX) on oligonucleosomal fragment accumulation induced by OA in primary rat hepatocytes. Cells were incubated with or without 20 nM OA for 8 hours, and when indicated a pretreatment with 20 or 50 µM SP600125, 10 µM JNKI1, 10 µM SB203580 or 100 µM cycloheximide was carried out. Accumulation of oligonucleosomal fragments in the cytoplasm was expressed as Enrichment Factor (EF), as described in Materials and Methods. Each bar represents the mean ± SD of quadruplicate determinations from at
least two independent experiments. (*p<0.05, a, vs control; b, vs OA-treated cells). **B**, Effect of SP600125 (SP) on caspase 3 activation induced by OA. Cells were pretreated with 50 µM SP600125 and incubated with 20 nM OA for 4 hours. Western blot was carried out using a specific antibody for the activated form of caspase 3 (p17 subunit).

**C**, Effect of SP600125 on AP-1 binding activity induced by OA in primary rat hepatocytes. Cells were pretreated with 50 µM SP600125 and incubated with 20 nM OA for 4 hours. EMSA was carried out using a radiolabelled consensus probe for AP-1.

**Figure 4.** Effect of pretreatment with AdoMet or MTA on primary rat hepatocytes incubated with OA. Primary rat hepatocytes were pretreated with AdoMet (4 mM) or MTA (500 µM) and treated with 20 nM OA for 4 hours. **A**, Western blot analysis of JNK, c-Jun, p38 and ATF-2 phosphorylation. Total protein levels were identified using specific antibodies, and their phosphorylation levels analyzed using antibodies for the phosphorylated forms. **B**, AP-1 binding activity analyzed by EMSA using a radiolabelled consensus AP-1 probe.

**Figure 5.** **A**, Analysis of JNK phosphorylation in HuH7 human hepatoma cells treated with okadaic acid. Cells were treated with 125 nM OA for the indicated times and JNK was identified in protein extracts by Western blot using specific antibodies for JNK and for the phosphorylated form of the enzyme. **B**, Effect of SP600125 (SP) on caspase 3 activation and c-Jun phosphorylation induced by OA. Cells were pretreated with 50 µM SP600125 and incubated with 20 nM OA for 4 hours. Western blot was carried out using a specific antibody for the activated form of caspase 3 (p17) and for p-c-Jun.
Figure 6. AP-1 binding activity in HuH7 human hepatoma cells treated with okadaic acid. Cells were treated for the indicated times with 125 nM OA and nuclear extracts obtained as described in Materials and Methods. EMSA was carried out using a radiolabelled consensus probe for AP-1. A, time course analysis of AP-1 binding activity. B and C, analysis of AP-1 components in control (B) or OA-treated cells (C). When indicated, cells were incubated with 125 nM OA for 4 hours and specific antibodies for different members of AP-1 were added to the reaction mixture. The supershifts obtained are shown with arrow-heads.

Figure 7. Effect of pretreatment with AdoMet or MTA on HuH7 cells incubated with OA. Cells were pretreated with AdoMet (4 mM) or MTA (500 µM) and treated with 125 nM OA for 4 hours. A, Western blot analysis of JNK, c-Jun, p38 and ATF-2 phosphorylation. Total protein levels were identified using specific antibodies, and their phosphorylation levels analyzed using antibodies for the phosphorylated forms. B, AP-1 binding activity analyzed by EMSA using a radiolabelled consensus AP-1 probe.
Figure 1
Figure 2

A

OA  | 1 h | 2 h | 3 h | 4 h |
---|---|---|---|---|
-  | +  | +  | +  | +  |

B

OA  | 1 h | 2 h | 3 h | 4 h |
---|---|---|---|---|
-  | +  | +  | +  | +  |

C

Antibody  | α-D-Jun | α-Jun B | α-Jun C | α-Fos |
---------|---------|---------|---------|--------|
OA  | -  | +  | +  | +  | +  |
Figure 3A
Figure 3B and C

B

Control OA SP SP + OA

Caspase 3 →

β actin →

C

Control OA SP SP + OA

AP-1 →
Figure 4A
Figure 5

A

<table>
<thead>
<tr>
<th></th>
<th>1 h</th>
<th>2 h</th>
<th>4 h</th>
<th>6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>OA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-JNK</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JNK</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>OA</th>
<th>SP</th>
<th>SP + OA</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-c-Jun</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caspase 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β actin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 6

A

OA

- + + +

2h 4h 8h

B

Antibody

α-P-α-Jun
α-Jun B
α-Jun D
α-Fos

OA

- - - -

C

Antibody

α-P-α-Jun
α-Jun B
α-Jun D
α-Fos

OA

- + + + +
Figure 7A
Figure 7B

[Image of a gel electrophoresis with lanes labeled Control, OA, AcO-Met, MTA, AcO-Met + OA, MTA + OA, and an arrow pointing to the AP-1 band]